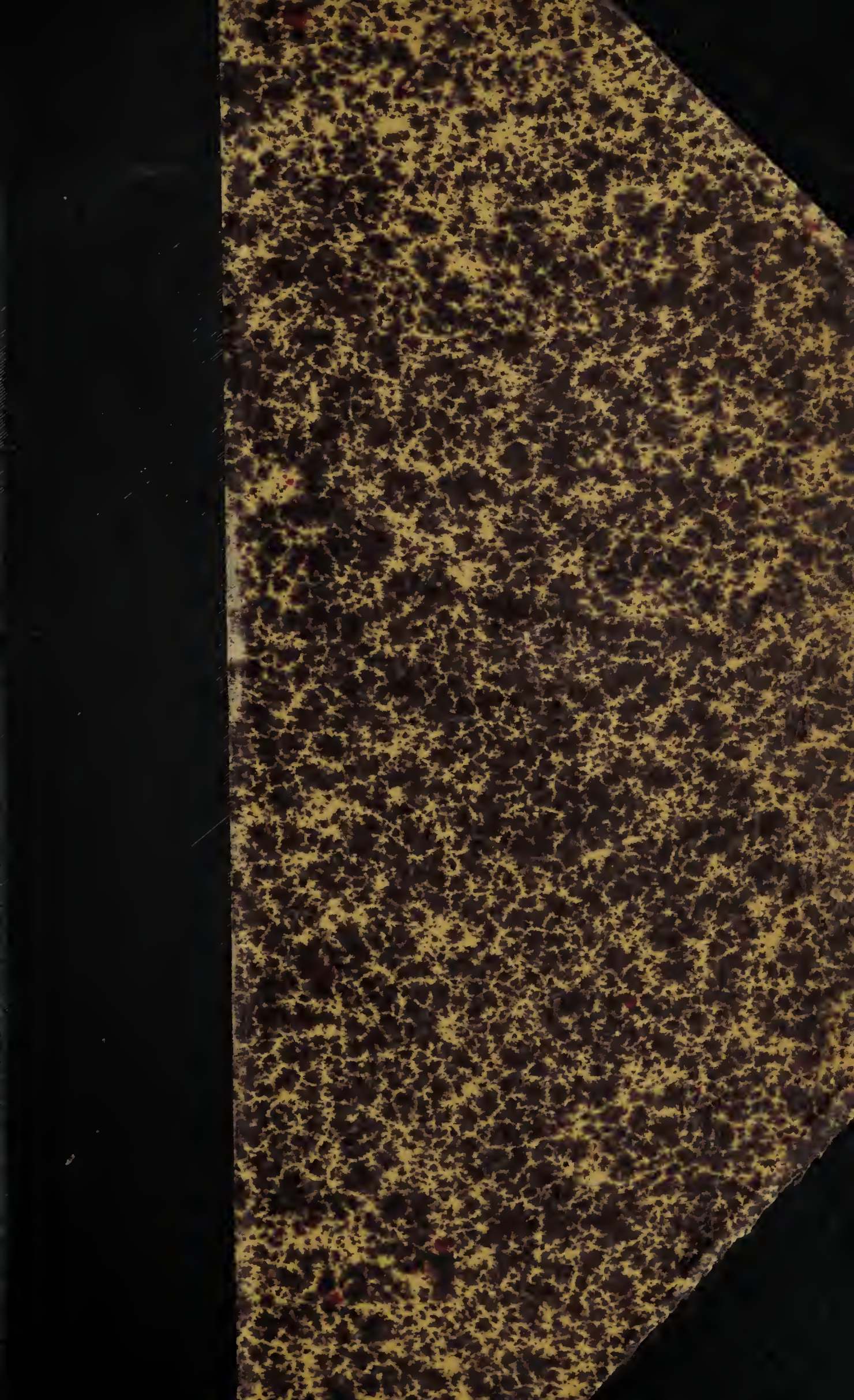




42-N53p v.21

1922

W. G. FARLOW

THE NEW PHYTOLOGIST

A BRITISH BOTANICAL JOURNAL

EDITED BY

A. G. TANSLEY, M.A., F.R.S.

UNIVERSITY LECTURER IN BOTANY
CAMBRIDGE

IN CONSULTATION WITH THE STAFF OF
THE CAMBRIDGE BOTANY SCHOOL

VOLUME XXI

PAGES viii AND 300

WITH NUMEROUS FIGURES
IN THE TEXT

LONDON

WHELDON & WESLEY, LTD.

2, 3 & 4, ARTHUR ST., NEW OXFORD ST., W.C. 2

1922

42

1453p

121

1722

INDEX OF SUBJECTS

	PAGE
Abietineæ, <i>see</i> Endodermis in the Leaves of the	
Algae, Freshwater, <i>see</i> Periodicity	
Anatomy, Physiological Studies in Plant—J. H. Priestley	
I. Introduction	58
II. The Physiological Relation of the Surrounding Tissue to the Xylem and its Contents—(with Dorothy Armstead)	62
III. The Structure of the Endodermis in Relation to its Function (5 Text-figs.)—(with Edith E. North) .	113
IV. The Water Relations of the Plant Growing Point (2 Text-figs.)—(with R. M. Tupper-Carey) .	210
V. Causal Factors in Cork Formation—(with Lettice M. Woffenden)	252
Autumnal or Second Flowerings of Plants, Records of—Sir Francis Darwin and A. Shrubbs	48
Bench Light, A cheap (Laboratory Note) (1 Text-fig.)—Sydney J. Mangham	231
Cell Wall, The, <i>see</i> Permeability	
Commutator, <i>see</i> Multiple Switch	
Cork Formation, Causal Factors in—(Physiological Studies in Plant Anatomy, V)—J. H. Priestley and Lettice M. Woffenden	252
Cyanophyceae from the point of view of their evolution, A critical study of certain unicellular—W. B. Crow (1 Text-fig.) .	81
Endodermis in Relation to its Function, The Structure of the (5 Text-figs.)—(Physiological Studies in Plant Anatomy, III)— J. H. Priestley and Edith E. North	113
Endodermis in the Leaves of the Abietineæ, The Structure and Function of the (12 Text-figs.)—Isabel Soar	269
Excretion of Water by Plant Cells, <i>see</i> Permeability	
Growing point, The Water Relations of the Plant (2 Text-figs.)— (Physiological Studies in Plant Anatomy, IV)—J. H. Priestley and R. M. Tupper-Carey	210
Hybrid, <i>see</i> <i>Primula elatior</i> × <i>vulgaris</i>	
Intake of Water by Plant Cells, <i>see</i> Permeability	

	PAGE
Laboratory Notes:	
(1) <i>See</i> Bench Light	231
(2) <i>See</i> Multiple Switch	166
(3) <i>See</i> Projection Microscope	163
(4) <i>See</i> Respiroscope	230
<i>Lichens</i> , by Annie Lorrain Smith [Review]—W. Watson	102
Multiple Switch and Commutator for Thermocouples, A (Laboratory Note) (1 Text-fig.)—H. J. Denham	166
Mycologists, Notices to—	
(1) The Lister Institute National Collection of Type Cultures	232
(2) Continuation of Saccardo's <i>Sylloge Fungorum</i>	232
Osmotic Pressure, <i>see</i> Permeability	
Periodicity of Freshwater Algae in Nature, A Study of some of the Factors controlling the—(concluded) (2 Text-figs.)—W. J. Hodgetts	15
Permeability—Walter Stiles	
VI. Osmotic Pressure	1
VII. The Cell Wall	49
VIII. The Plasma-Membrane	140
IX. The Water Relations of the Plant Cell	169
X. The Influence of External Conditions on the Intake and Excretion of Water by Plant Cells and Tissues	233
Phænology, Studies in, No. 3 (1921) (with Tables)—Sir Francis Darwin	34
Plasma-Membrane, The, <i>see</i> Permeability	
<i>Primula elatior</i> × <i>vulgaris</i> , The origin of the hybrid, demonstrated experimentally in the field, with notes on other British <i>Primula</i> hybrids—Miller Christy	293
<i>Primula vulgaris</i> , <i>see</i> <i>Primula elatior</i>	
Projection Microscope, The (Laboratory Note)—H. J. Denham	163
Protoplasmic Streaming, A method for inducing—William Seifriz	107
Respiroscope, A Simple (Laboratory Note)—Sydney J. Mangham	230
Root Pressure, Further Observations upon the Mechanism of—J. H. Priestley	41
Second or Autumnal Flowerings of Plants, Records of—Sir Francis Darwin and A. Shrubbs	48
<i>Sylloge Fungorum</i> , Continuation of Saccardo's—(Notices to Mycologists)	232

Index of Subjects

V

PAGE

Thermocouples, *see* Multiple Switch

Type Cultures, The Lister Institute National Collection of—
(Notices to Mycologists) 232

Water, Intake and Excretion of, *see* Permeability

Water Relations of the Plant Cell, The, *see* Permeability

Xylem and its Contents, The Physiological Relation of the Sur-
rounding Tissue to the—(Physiological Studies in Plant Ana-
tomy, II)—J. H. Priestley and Dorothy Armstead 62

INDEX OF AUTHORS

	PAGE
ARMSTEAD, DOROTHY, <i>see</i> PRIESTLEY	
CHRISTY, MILLER. The origin of the hybrid <i>Primula elatior</i> × <i>vulgaris</i> demonstrated experimentally in the field, with notes on other British <i>Primula</i> hybrids	293
CROW, W. B. A critical study of certain unicellular Cyanophyceæ from the point of view of their evolution (1 Text-fig., 2 Tables)	81
DARWIN, Sir FRANCIS. Studies in Phænology, No. 3 (1921)	34
DARWIN, Sir FRANCIS and SHRUBBS, A. Records of Autumnal or Second Flowerings of Plants	48
DENHAM, H. J. A multiple switch and commutator for thermocouples (Laboratory Note) (1 Text-fig.)	166
— The projection microscope (Laboratory Note) (1 Text-fig.)	163
HODGETTS, WILLIAM J. A study of some of the factors controlling the periodicity of freshwater algae in nature (<i>concluded</i>) (2 Text-figs.)	15
MANGHAM, SYDNEY J. A simple respiroscope (Laboratory Note)	230
— A cheap bench light (Laboratory Note)	231
NORTH, EDITH E., <i>see</i> PRIESTLEY	
PRIESTLEY, J. H. Further observations upon the mechanism of root pressure	41
— Physiological studies in plant anatomy. I. Introduction	58
PRIESTLEY, J. H. and ARMSTEAD, DOROTHY. The physiological relation of the surrounding tissue to the xylem and its contents (Physiol. studies in plant anatomy, II) (3 Tables)	62
PRIESTLEY, J. H. and NORTH, EDITH E. The structure of the endodermis in relation to its function (Physiol. studies in plant anatomy, III) (5 Text-figs.)	113
PRIESTLEY, J. H. and TUPPER-CAREY, R. M. The water relations of the plant growing point (Physiol. studies in plant anatomy, IV) (2 Text-figs.)	210
PRIESTLEY, J. H. and WOFFENDEN, LETTICE M. Causal factors in cork formation (Physiol. studies in plant anatomy, V)	252

Index of Authors

vii

	PAGE
SEIFRIZ, WILLIAM. A method for inducing protoplasmic streaming	107
SHRUBBS, A., <i>see</i> DARWIN	
SOAR, ISABEL. The structure and function of the endodermis in the leaves of the Abietineæ (12 Text-figs.)	269
STILES, WALTER. Permeability	
VI. Osmotic Pressure (3 Text-figs.)	1
VII. The Cell Wall (1 Table)	49
VIII. The Plasma-Membrane	140
IX. The water relations of the plant cell (8 Tables)	169
X. The influence of external conditions on the intake and excretion of water by plant cells and tissues	233
WATSON, W. Review of <i>Lichens</i> by Annie Lorrain Smith	102
WOFFENDEN, LETTICE M., <i>see</i> PRIESTLEY	

THE NEW PHYTOLOGIST

VOL. XXI, No. 1

MARCH 22, 1922

PERMEABILITY

By WALTER STILES

CHAPTER VI

OSMOTIC PRESSURE

THE diffusion of a liquid through a membrane is termed osmosis. As long ago as 1748 it was observed by Nollet that when a bladder filled with alcohol was immersed in water the bladder swelled, while when a bladder full of water was immersed in alcohol the bladder shrank owing in each case to the water passing through the membrane more rapidly than alcohol.

Similarly, if an aqueous solution of, say, sucrose is contained in a bladder impermeable to the solute but permeable to the solvent, on immersing the bladder in water, the latter alone passes through the membrane into the bladder; which is in consequence distended, and may indeed ultimately burst if the solution inside is sufficiently concentrated. Thus when a pure solvent and solution are separated by a semi-permeable membrane, the pure solvent tends to pass through the membrane so as to dilute the solution, for this is the only way in which diffusion can take place so as to bring about equalisation of concentration throughout the system. Consequently when solution and pure solvent are separated by a semi-permeable membrane a hydrostatic pressure is produced which forces water through the membrane from solvent to solution.

The osmotic pressure of a solution is defined as "the equivalent of the hydrostatic pressure produced when the solution and solvent are separated by a perfectly semi-permeable membrane; or as the equivalent of the excess pressure which must be imposed on a solution in order to prevent the passage into it of solvent through a perfectly semi-permeable membrane" (Findlay, 1913).

DETERMINATION OF OSMOTIC PRESSURE

Osmotic pressure is easily demonstrated by the familiar laboratory experiment in which a solution of sucrose is placed in an inverted thistle funnel, the mouth of which is closed by a parchment membrane and which is then placed in a vessel of pure water. The membrane being impermeable to sucrose, water passes through the membrane and consequently the level of liquid rises in the tube of the thistle funnel. This process continues until the pressure of the height of liquid in the funnel is equal to the osmotic pressure forcing water into the funnel. The principle involved in this experiment furnishes the basis for the direct method of measuring osmotic pressure. For a detailed description of this method and of the indirect methods of measuring the osmotic pressure of a solution, reference may be made to the monograph by Findlay already cited. Here it will be sufficient to record the various methods which are employed. These are as follows:

1. *Direct Method.* In the direct method of measuring osmotic pressure a semi-permeable precipitation membrane of copper ferrocyanide is deposited in the wall of a porous pot into the mouth of which is sealed a manometer which serves for the determination of the pressure produced. Great difficulties are involved in this method, and the original apparatus devised by Pfeffer (1877) has since been greatly improved by later workers, especially by Morse and his co-workers (1901-1912) and by Berkeley and Hartley (1904, 1906 a).

The indirect methods of determining osmotic pressure depend on the relations which exist between osmotic pressure and various physical properties. According to the particular property measured the indirect methods may be called the vapour pressure method, the freezing point method, the boiling point method, and the critical solution temperature method.

2. *Vapour Pressure Method.* The vapour pressure of a liquid is lowered by the presence of a dissolved substance. A number of equations have been obtained by different investigators for the relation between vapour pressure and osmotic pressure. The formula of Spens (1906) is probably an exact enough approximation for most purposes. This equation is

$$Pv_s = sp \log_e \frac{p}{p'},$$

where P is the osmotic pressure, p and p' are the vapour pressures

of pure solvent and solution respectively, s the specific volume of the vapour and v_s is the increase in volume of a large mass of solution on addition of unit mass of solvent. For other formulæ, more or less exact, Findlay's book may be consulted, or the original papers of Arrhenius (1889), Berkeley and Hartley (1906 *b*), Porter (1907, 1908), Callendar (1908), and Berkeley, Hartley and Burton (1908). The actual method of measurement of the vapour pressure depends on bubbling an indifferent gas through the solution, the amount of vapour taken up by the gas being proportional to the vapour pressure. In the original experiments of Walker (1888) the gas is allowed to bubble first through the solution and then through the pure solvent. The amount of vapour taken up in the second operation is proportional to the difference between the vapour pressures of pure solvent and solution.

3. *Freezing Point Method.* As is well known the presence of a dissolved substance lowers the freezing point of a solution. A number of formulæ have been derived also in this case to express the relation between lowering of freezing point and osmotic pressure, for a discussion of which the reader is again referred to Findlay.

4. *Boiling Point Method.* Just as determinations of the lowering of the freezing point of a solution due to a dissolved substance can be used to calculate the osmotic pressure of a solution, so the elevation of the boiling point resulting from the presence of a solute can be used to the same end. The method has, however, been little employed for this purpose.

5. *Critical solution temperature method.* Certain liquids are miscible in all proportions only above a certain temperature. Below this temperature they are miscible only to a limited extent, and consequently if a liquid is cooled below this temperature one of the liquids separates out as a distinct phase. This critical solution temperature is altered by dissolved substances in proportion to the osmotic pressure of the solute. A method of measuring osmotic pressure based on the work of Timmermans (1907) in this relation has been employed for physiological purposes by Atkins and Wallace (1913) to whose work further reference may be made.

Methods for *comparing* osmotic pressures by the use of living cells and tissues were devised by de Vries (1884 *a, b*). These are the plasmolytic methods and the tissue tension method. They will be more conveniently described in a later chapter dealing with the water relations of the plant cell.

RELATION OF OSMOTIC PRESSURE TO TEMPERATURE AND CONCENTRATION

The osmotic pressure of an aqueous solution depends on the dissolved substance, its concentration and the temperature. In the case of dilute solutions, Pfeffer (1877) concluded that the osmotic pressure

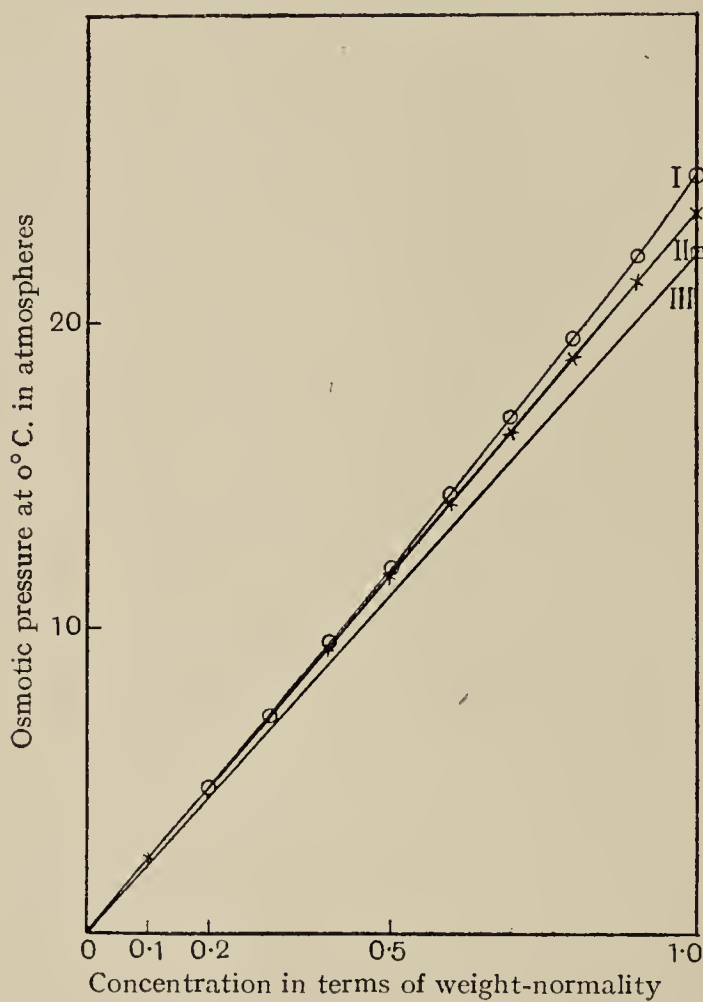


Fig. 4. Relation between concentration of a solution and its osmotic pressure. I. Measured values for sucrose. II. Measured values for glucose. III. Theoretical values calculated from the equation $PV = nRT$. (Data from Morse and collaborators.)

is proportional to the absolute temperature and to the concentration, that is,

$$P = K \cdot CT,$$

where P is the osmotic pressure, C the concentration and T the absolute temperature.

Now the concentration is the reciprocal of the volume occupied by unit mass of the solute, so that the relation becomes similar in

form to that connecting pressure, volume and temperature of a gas, namely

$$Pv = KT.$$

Van't Hoff (1887) further showed that the equation is not merely the same but identical with the gas law equation and that if v is measured as the volume of one gram-molecule, then K has the same value as the gas constant R in the equation

$$Pv = RT,$$

which in consequence holds also for dilute solutions.

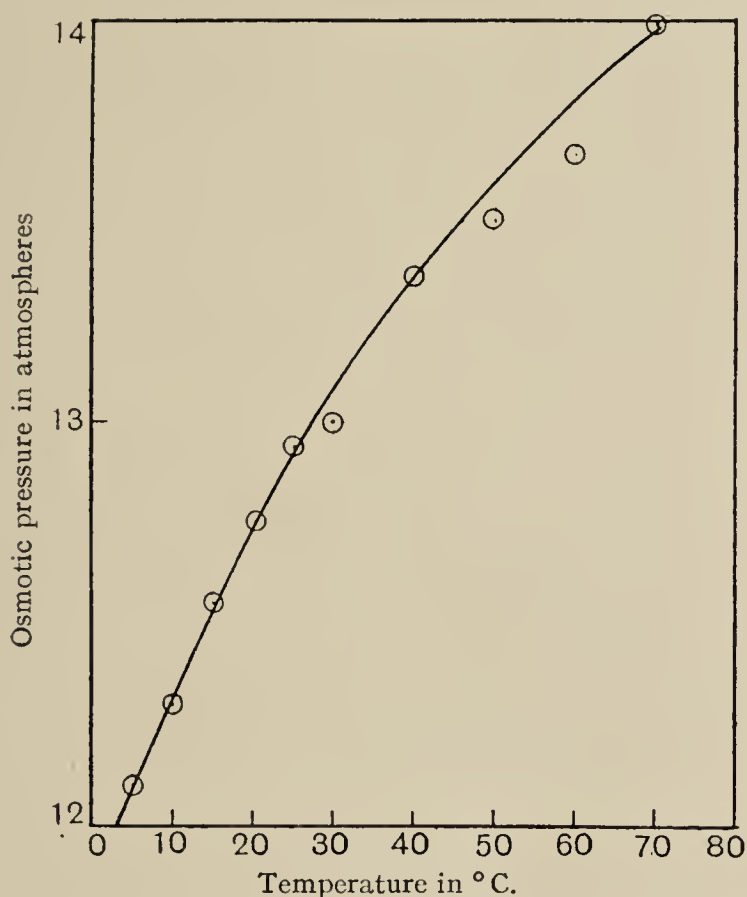


Fig. 5. Relation between temperature and osmotic pressure of a 0.5 weight normal solution of sucrose.

If V is the volume of the solution and n the number of gram molecules of solute present in the volume V , the equation becomes in consequence

$$PV = nRT.$$

How near an approximation this is in dilute solutions will appear from Figs. 4 and 5. In Fig. 4 is shown in graphical form the relation between concentration of solutions of sucrose and osmotic pressure at constant temperature, and in Fig. 5 is shown the relation between

temperature and osmotic pressure at constant concentration in the case of sucrose. These curves are constructed from the figures provided by Morse and his co-workers. For a large mass of further data reference may be made to the original papers of these workers.

It will be observed that in concentrations up to normal the proportionality between concentration and osmotic pressure holds quite well, but it is to be noticed that the measured osmotic pressure is always higher than that calculated from the gas law. This difference

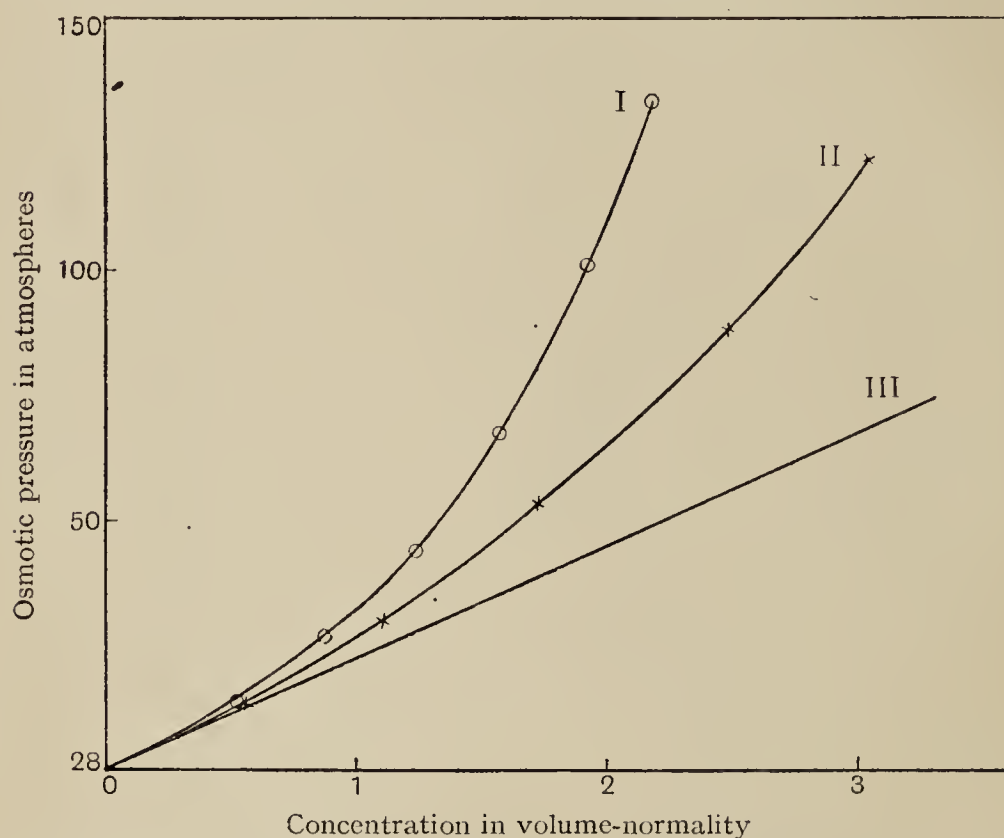


Fig. 6. Relation between concentration and osmotic pressure of solutions of sucrose and glucose. I. Sucrose at 0°C . II. Glucose at 0°C . III. Theoretical curve at 0°C . (Data from Berkeley and Hartley.)

between observed and calculated values is greatest at the higher concentrations, and in very high concentrations this divergence increases, as the curves in Fig. 6, constructed from the data obtained by Berkeley and Hartley, clearly show. The relation between temperature and osmotic pressure is also not one of strict proportionality over large temperature intervals. It has been pointed out by Morse that the divergence between observed and calculated values is considerably lessened even at high concentrations if it is assumed that the osmotic pressure is equal to the pressure the solute would exert

as a gas occupying the volume of the solvent only and not the volume of the solution. Even so, the divergence is not eliminated, and consequently various formulæ have been put forward to relate more exactly the concentration, temperature and osmotic pressure of solutions. A consideration of these is outside the scope of this work, and the reader is referred to the authorities already cited. It may, however, be emphasized that the simple van't Hoff law is valid for dilute solutions.

It has been pointed out that the deviations of concentrated solutions of sucrose from the behaviour of "ideal" solutions may to a large extent be explained on the assumption that sucrose is hydrated in solution, that is, that with each molecule of sugar a certain number of molecules of water become associated. In concentrated solutions this may materially reduce the quantity of solvent and so increase the concentration of solute, and consequently the osmotic pressure, in the way actually observed. In dilute solutions, where the quantity of solvent is very large in proportion to the quantity of solute the influence on concentration of solute is negligible (Nernst, 1911; Findlay, 1913).

MEASURED VALUES OF THE OSMOTIC PRESSURE OF SUCROSE.

A consideration of the work of Berkeley and Morse and their respective collaborators is sufficient to indicate the very great care and persistence required for the exact determination of osmotic pressures. Fortunately the substance most carefully studied, namely, sucrose, is one of particular importance for the student of permeability in plants, as will appear in a later chapter. It will therefore be useful to record here a few of the values obtained by Morse and regarded by him as established with a reasonable degree of certainty. In the following table are therefore given the values for the osmotic pressure of sucrose in different concentrations at temperatures most generally encountered in working with plants. For values at other temperatures, as well as for values of the osmotic pressure of solutions of glucose and mannite reference must be made to the summary of his work made by Morse (1914).

TABLE XV
Osmotic Pressures of Aqueous Solutions of Sucrose.
(Data from Morse)

Concentration in weight- normalities	Osmotic pressure at various temperatures			
	0° C.	10° C.	20° C.	30° C.
0.1	2.462	2.498	2.590	2.474
0.2	4.723	4.893	5.064	5.044
0.3	7.085	7.335	7.605	7.647
0.4	9.443	9.790	10.137	10.295
0.5	11.895	12.297	12.748	12.978
0.6	14.381	14.855	15.388	15.713
0.7	16.886	17.503	18.128	18.499
0.8	19.476	20.161	20.905	21.375
0.9	22.118	22.884	23.717	24.226
1.0	24.826	25.693	26.638	27.223

OSMOTIC PRESSURE OF ELECTROLYTES

So far this discussion has been confined to sugars and the remarks already made may be regarded as applying generally to crystalloidal non-electrolytes. There are, however, two other groups of substances of the greatest importance which require some special treatment; these are electrolytes and colloids.

In the case of electrolytes the osmotic pressure is found to be considerably higher than the theoretical pressure calculated from the gas law. This is correlated with the dissociation of the molecule into its constituent ions. If each particle, either ion or molecule, is regarded as exercising the same osmotic pressure then agreement with the general law is found to exist in the case of dilute solutions of electrolytes as well as dilute solutions of non-electrolytes.

The formula

$$PV = nRT$$

must therefore be modified in the case of electrolytes to

$$PV = n (\alpha\beta + 1 - \alpha) R \cdot T,$$

where α is the degree of dissociation of the electrolyte and β the number of ions into which the molecule dissociates.

Electrolytes have not so far received the attention that has been devoted to sucrose with regard to exact measurement of the osmotic pressure. The investigation of electrolytes is fortunately being undertaken by Morse and some results have already been obtained with lithium chloride. The values obtained by de Vries for a number of electrolytes and non-electrolytes will be referred to in a later chapter.

OSMOTIC PRESSURE OF COLLOIDS

Whether colloids exert an osmotic pressure or whether they do not is a question which has been the subject of some discussion. Now as colloidal properties are due either to the large size of the molecule, or to the aggregation of a number of molecules, we should expect colloids to exhibit the same behaviour in regard to osmotic pressure as other substances. The influence of association of molecules in the case of hydration has already been touched upon.

It was Starling (1896, 1899) who first showed experimentally that a colloid exerted an osmotic pressure. Blood serum was filtered through gelatine under pressure, and in this way the crystalloids were separated from the colloids, which alone were held back. The filtrate was then separated from unfiltered serum by a gelatine membrane, and the hydrostatic pressure developed was measured; any osmotic pressure produced is then attributable to the colloids. In this way it was shown that the colloids of blood serum exerted an osmotic pressure of 3 to 4 cm. of mercury. Further determinations of the osmotic pressure of colloids have since been made by other observers, notably by Hüfner and Gansser (1907) who found the osmotic pressure of hæmoglobin corresponds to the molecular weight calculated from chemical data (cf. also Reid, 1905), and it may now be assumed with confidence that colloids do actually exert a definite osmotic pressure. Owing to the large size of the molecule or molecular association this osmotic pressure will naturally be small as compared with that produced by equal weight of a crystalloid.

THEORIES OF OSMOTIC PRESSURE

Explanations that have been put forward of the origin of osmotic pressure fall into two groups. In the first group osmotic pressure is supposed to arise in a similar way to gas pressure by the bombardment of the molecules (or ions) of the solute upon the separating membrane.

This theory may be called the kinetic theory of osmotic pressure. It was first put forward as a possible explanation by van't Hoff and is suggested at once by the fact that the osmotic pressure of dilute solutions is equal to the pressure the solute would exert as a gas occupying a volume equal to that of the solution. The contrasted hydrostatic theory, due in the first place to Jäger (1891 *a, b*) finds an origin for osmotic pressure in the different surface tensions of solution and pure solvent. This view, as elaborated by Moore (1894) regards

osmotic pressure as due to the movement of liquid through the capillary spaces of the semi-permeable membrane from the liquid of lower surface tension to that of higher surface tension. Modifications of the theory have been proposed by I. Traube (1904) and Battelli and Stefanini (1905, 1907 *a, b, c*) a presentation of which is outside the scope of the present work. For an adequate discussion of the views of these and other writers the reader is referred to Findlay's monograph already cited.

NEGATIVE OSMOSIS

In all the cases so far discussed in which a solution is separated from the solvent by a membrane, there results a passage of liquid across the membrane from solvent into solution. It is now necessary to turn to a phenomenon which appears anomalous and inexplicable by the laws so far considered. This is the phenomenon of *negative osmosis* characterised by the flow of liquid across the membrane from the solution to the pure solvent.

Negative osmosis was observed by Dutrochet (1827) and later by T. Graham (1854). The phenomenon has recently formed the subject of a series of investigations by Girard, working with membranes of pig's bladder, and by Bartell and his collaborators, who employed membranes of porcelain and gold-beaters' skin. The results of these investigations seem likely to have important bearings on questions of cell permeability, so that some detailed consideration of them appears necessary.

Bartell (1914) first worked with membranes of porcelain about 5 mm. thick, the pores of which were about 0.2μ in diameter. This diameter of pore is on the border-line as regards osmotic effects; with somewhat wider pores osmotic effects are not produced, while with narrower pores the osmotic effects are definite. The membranes are thus decidedly permeable to dissolved substances.

Osmotic cells were prepared in which the separating membrane was porcelain, and the cells were filled with solutions of the salts examined. These included chlorides, nitrates, sulphates and acetates of a number of monovalent and divalent kations in concentrations of $M/10$ and, in the case of sulphates, $M/5$ and $M/2$. In all the experiments in which potassium and ammonium salts were employed passage of liquid took place in the normal direction, that is, from the solvent to the solution (positive osmosis), with sodium sulphate and sodium acetate positive osmosis also occurred, as it did also in experiments with lithium sulphate; on the other hand, the chlorides and

nitrates of sodium, lithium, zinc, manganese and magnesium, the sulphates of the last three named metals, and the acetates of zinc and manganese all gave negative osmosis. Confirmatory results were obtained by using pure water in the cell and solutions outside.

It is important to observe that the membranes employed are not semi-permeable, and indeed the incidence of negative osmosis is related to the diameter of the pores in the membrane. Thus with $M/10$ magnesium chloride solutions, and with porcelain membranes, no apparent osmotic effects were produced when the diameter of the pores was greater than 0.4μ , with pore diameters between 0.4μ and 0.1μ negative osmosis was observed, while with pore diameters less than 0.1μ osmosis was in the positive direction as with semi-permeable membranes.

Further observations were made by Bartell and Hocker (1916 *b*) on the osmosis of the following solutions: (1) nitrates of a number of univalent (K, NH_4 , Na, Li), bivalent (Ba, Zn, Mn, Mg), trivalent (Al) and tetravalent (Th) kations in concentrations varying from $0.0005 M$ to M ; (2) $0.1 M$ solutions of a number of potassium salts (NO_3 , Cl, Br, I, CNS, acetate, SO_4 , CrO_4 , PO_4 , ferrocyanide and ferricyanide); (3) hydrochloric acid and sodium hydroxide in concentrations from $0.001 M$ to $0.2 M$, and (4) $0.1 M$ solutions of some nitrates when the membranes were immersed in solutions of acids and alkalis in different concentrations.

The results obtained were very varied. In some cases positive osmosis occurred in all concentrations, increasing regularly with increase in concentration, in others positive osmosis occurred in low concentrations but decreased with increasing concentration so that in higher concentrations negative osmosis occurred. In other cases there was observed a definite concentration for maximum positive osmosis which decreases with either higher or lower concentrations, while with yet other electrolytes there is a concentration for maximum negative (or minimum positive) osmosis, all concentrations above or below this value giving more positive effects.

More recently Bartell and Madison (1920 *a*) have shown that abnormal osmosis also occurs with membranes of gold-beaters' skin, the flow of water across the membrane being sometimes in the direction of solvent to solution, sometimes in the reverse direction. In the case of normal (positive) osmosis, the flow, as compared with that into a solution of sucrose, was sometimes abnormally large and sometimes abnormally small. The same authors further show (1920 *b*)

that the osmosis of salt solutions is markedly affected by the presence of acids and alkalies.

The explanation of negative osmosis put forward by Girard (1908, 1909, 1910 *a*, 1910 *b*, 1914) and also by Bartell and his collaborators (1914, 1916 *a*, 1920 *a*, *b*) ascribes the phenomenon to the action of electrical forces. It has already been noted (Chapter III) that at the surface of separation of two liquids there exists a potential difference. But Bartell and Madison quote the results of Brünings (1903, 1907), Lillie (1911), Beutner (1913 *e*), Girard and Bartell and Hocker to show that permeable membranes of almost any material separating water and a solution exhibit a difference of potential on the two sides of the membrane which is different from the potential difference arising from contact of the two liquids. Girard supposes that in addition to the difference of potential between the two surfaces of the membrane there is also an electric charge on the walls of the capillaries of the membrane, while the liquid in the capillaries possesses a charge opposite to that on the walls of the capillaries, there being produced a Helmholtz double layer (Helmholz, 1879). This charge on the walls of the capillaries is supposed to be determined by the preponderance of a small excess of hydrogen or hydroxyl ions as suggested by Perrin (1904). Such a state of affairs would tend to the movement of negatively charged water, for example, in the direction of the surface of the higher potential, and *vice versa*.

The explanation of negative osmosis offered by Bartell and his collaborators is essentially similar. The tendency to normal osmosis, determined by the differences in salt concentration on the two sides of the membrane will always be present, but superimposed on these is the passage of water brought about by the presence of electrical factors. These are as Girard suggested (1) the electric charge on the membrane with respect to the liquid layer in the capillaries of the membrane, that is, the value of the Helmholtz double layer between membrane and liquid, and (2) the polarisation of the membrane, that is, the difference of potential between the two faces of the membrane. The value of the former we have noted is ascribed by Girard to the influence of an excess of hydrogen or hydroxyl ions. Bartell and his collaborators throw doubt on the adequacy of this explanation and suppose three factors to be active here, namely (*a*) the extent of the diffusion of the electrolyte through the membrane (phase potential), (*b*) the relative migration velocities of the ions (diffusion potential), and (*c*) the extent of selective adsorption

by the membrane (adsorption potential). These three ways in which potential differences may arise have been described in Chapter III.

There are nine possible cases of electrification, and these are clearly set out in diagrammatic form by Bartell and Madison. These are here shown in tabular form in Table XVI.

TABLE XVI
Possible Cases of Electrification of Membranes and
the Effect on Osmosis

No.	Charge on solution side of membrane	Charge on liquid in capillaries	Osmosis
1	None	None	Normal
2	None	+	Normal
3	None	-	Normal
4	+	None	Normal
5	+	+	Abnormally low
6	+	-	Abnormally high
7	-	None	Normal
8	-	+	Abnormally high
9	-	-	Abnormally low

It is to be observed that all these expected results have been realised in practice, as the summary of Bartell's work already given here has indicated. In cases 5 and 9, where we have respectively positively charged water moving to the side of the membrane negatively charged, and *vice versa*, the tendency for liquid to move from the solution into the solvent may exceed the tendency for normal osmosis to take place, in which case negative osmosis results. It is to be noted that both Girard (1919) and Bartell and Madison (1920) measured the potential difference between the two sides of the membranes they employed, and have shown that this is such as the theory requires. It is also possible that the electrification of the system might arise in other ways (cf. Freundlich, 1916). The phenomenon, it will be observed, is similar to that of electrical osmosis, in which water passes from one solution to another across a membrane as a result of a potential difference applied to the solutions on either side of the membrane. (See especially Briggs, 1919).

A curious case of negative osmosis has recently been noted by Loeb (1920 *a*). When solutions of aluminium chloride or of lanthanum salts are separated from water by a collodion membrane treated with gelatine, water diffuses into the solution, although such diffusion does not take place when an untreated membrane is used. If such

an untreated collodion membrane separates water and a solution of an acid in concentration less than $M/16$, positive osmosis occurs, the more rapidly the higher the valency of the anion. When, however, a similar membrane treated with gelatine is used, negative osmosis takes place. Similar results were observed with membranes treated with casein, edestin, egg-albumin and serum albumin, but not with peptone, alanine or starch. These results are attributed to electrical forces which in concentrations of solutions below $M/16$ mainly determine the osmotic effects. In passing through the untreated collodion membrane the water is positively charged; on treating the membrane with gelatine the charge on the water becomes negative, so that the electrical forces will tend to send the water in the opposite direction. Further evidence for, and elaboration of, this view is contained in a series of papers by Loeb (1920 *b, c, d, e, f, g*, 1921) to which further reference may be made. Enough has already been said here to indicate the importance in connection with osmosis of electrical phenomena associated with membranes. That such phenomena are present in the living animal cell has already been indicated by Girard (1919) and Girard and Morax (1920).

(To be continued)

A STUDY OF SOME OF THE FACTORS CONTROLLING THE PERIODICITY OF FRESHWATER ALGÆ IN NATURE

By WILLIAM J. HODGETTS, M.Sc.

(With 2 figures in the text)

XVII. THE VOLVOCALES

THE periodicities of four members of the Volvocales have been worked out, namely *Chlamydomonas Reinhardi*, *Pandorina Morum*, *Eudorina elegans*, and *Volvox aureus*. The first and last have their periodicities given in the table below, while *Pandorina* and *Eudorina* have their frequency-curves represented in Fig. 10. All these species, when present, generally had a scattered distribution in the pond, not being confined to any particular region.

Table showing the Occurrence of *Chlamydomonas Reinhardi* and *Volvox aureus* in Hawkesley Hall Pond during 1918-1921.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Chlamydomonas Reinhardi</i>	1918	—	—	✓	—	vr	r	r	rr	c	vr	—	—
	1919	—	—	—	—	vr	vr	vr	r	rc	vr	r	r
	1920	—	vr	vr	—	—	—	vr	r	rr	vr	vr	—
	1921	r	vr	vr	vr	rr	r						
<i>Volvox aureus</i>	1918	—	—	—	rr	rr!	—	—	—	r	vr	vr	rr
	1919	vr	r	vr	vr	rr!	—	—	—	—	—	—	—
	1920	—	vr	vr	rc	c!	vr!	—	—	—	—	—	—
	1921	—	—	—	—	—	—						

Of the three species of *Chlamydomonas* (*C. Reinhardi*, *C. Debaryana*, *C. monadina*) which were identified with certainty in the pond only one (*C. Reinhardi*) has been at all abundant, the other two being observed only occasionally, and were never present in any quantity. The above table thus practically gives the frequency of the genus as a whole in the pond. Three out of the four maxima shown by *C. Reinhardi* (cf. table above) fall rather curiously in the month of September, and in each of the three complete years development was practically confined to late summer and early autumn, while in both 1918 and 1919, the maximum occurred immediately after the

hottest month (August) of the year. A falling temperature (between 16.5° and 13° C.—mean monthly temperatures) appears to be more favourable than a rising one.

The species also appears to prefer a falling concentration of the water, growth usually taking place with a high or rather high concentration, decreasing between 27 and 18 degrees; and in this respect the slight development during October—November 1919, when the concentration was extremely high (see Fig. 3), is noteworthy. It seems probable (as certain workers have demonstrated by cultural experiments in the case of other species of *Chlamydomonas*¹) that *C. Reinhardi* is partly able to utilise directly soluble organic compounds, such as were abundant in this pond (cf. Section IV) more especially during times of high concentrations—in other words that the species prefers a mixotrophic mode of nutrition. There is no evidence that abundant sunshine is necessary for the development of this species.

C. Debaryana was observed in the pond only in November and December 1919, when it was “rather rare,” becoming very rare in January 1920. Its development coincided with a low average temperature, but with a very high concentration of the water (cf. Fig. 3), since between November 22nd and December 13th 1919 the concentration was falling from 30 to 24 degrees. This species, therefore, like *C. Reinhardi*, is probably adapted to a mixotrophic nutrition in nature. The same applies to *C. monadina* which was also noted in the pond in November (very rare) and December (rare) 1919, but not at any other time. *C. monadina* is undoubtedly a cold-water form, and has frequently been observed in small ponds and puddles in the midland counties, and always shows a pronounced development during late autumn and winter; while it appears to be favoured by the presence of organic matter in the water.

Pandorina Morum (Fig. 10) commences its development in the pond during April or May, with the rising spring-temperature, persists in more or less abundance throughout the summer, finally becoming very rare or disappearing altogether during September. Even the high summer-temperatures do not appear to affect this alga adversely (July 1918). The fluctuation of the species between May and August, however, is rather puzzling, and does not appear to be directly related either to the temperature or sunlight, although

¹ Cf. in this connection the work of Artari on *Chlamydomonas Ehrenbergii* (*Jahrb. f. wiss. Bot.* 52, 1913, pp. 410–466). Artari found that this species preferred a mixotrophic mode of nutrition in cultures.

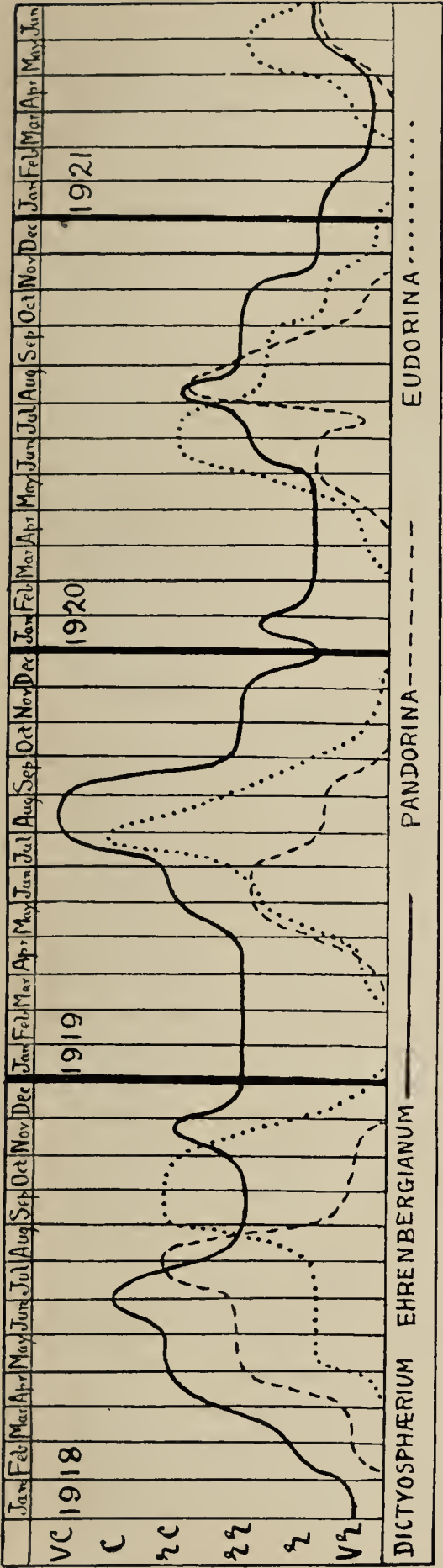


Fig. 10. Frequency-curves of *Dictyosphaerium Ehrenbergianum*, *Pandorina Morum*, and *Eudorina elegans*.

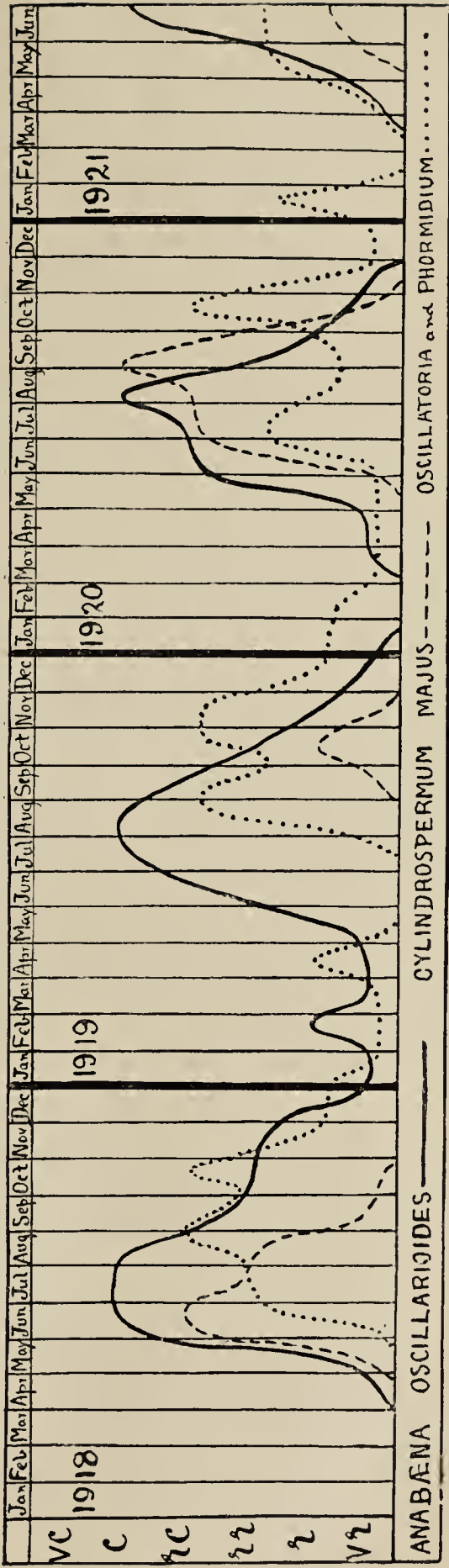


Fig. 11. Frequency-curves of the chief Blue-green Algae in Hawkesley Hall pond.

it shows some relation to the concentration of the water, while competition with other species (cf. *Eudorina* below) is probably a very important factor.

With regard to the concentration of the water the most favourable conditions appear to be when the concentration is gradually rising between 16 and 20 degrees, since a pronounced rise in the frequency-curve of the species (e.g. in July 1918, May 1919, and July—August 1920) always coincides with these concentration-conditions. In July and August 1919 concentrations above 20 degrees (cf. Fig. 3) were probably the cause of *Pandorina* becoming rare comparatively early in the year, although competition with *Eudorina* (or the attacks of aquatic animals) may also have had something to do with bringing about this result.

Eudorina elegans (Fig. 10) tends to commence its development in May, at about the same time as *Pandorina*, but in contrast to the latter species it may persist in quantity for some time after the decrease of *Pandorina* in early autumn, and thus appears to be a rather more hardy form. A comparison of the curves of *Pandorina* and *Eudorina* (Fig. 10) is rather interesting, and shows that it seems impossible for these two algæ to attain any considerable development in the pond at the same time¹. In 1920, for example, the increase in *Eudorina* during May and June apparently caused *Pandorina* to remain rare, but when *Eudorina* decreased in July the *Pandorina* suddenly became "rather common" (in early August). Again during June—July 1919 a rise in the *Eudorina*-curve involved a fall in that of *Pandorina*; and in August 1918 a fall in the *Pandorina*-curve apparently enabled *Eudorina* to develop. This appears to be evidence of the existence of mutual competition between these two algæ, such competition, however, acting adversely upon one of them only when the other has attained a fair degree of development in the pond. The only other explanation appears to be that the two species are favoured by a different set of external conditions, and that the conditions which favour one species exclude the pronounced growth of the other.

From a comparison of the various curves for 1919 and 1920 it might be deduced that *Eudorina* is favoured by a somewhat higher concentration of the water than *Pandorina*², but the evidence in 1918 points to exactly the opposite conclusion. Another point is that

¹ The same thing is apparent from the frequency-data of *Pandorina* and *Eudorina* given by Fritsch and Rich (18) in their study of Barton's pool.

² A conclusion arrived at by Fritsch and Rich (18) in the case of these two species in Barton's pool.

although *Eudorina* developed apparently equally well with either a falling or a rising concentration, pronounced growth of *Pandorina* always coincided with a rising and never a falling concentration. This may or may not be important, but on the whole it appears that mutual competition best explains the observed facts; and thus, in accordance with the general rule enunciated at the end of Section XII, little relation to the meteorological data, and to the concentration of the water, is apparent.

Volvox aureus has its occurrence in the pond given in the table at the beginning of this section, from which it is obvious that the species is a pronounced spring-form, generally developing during March and April, culminating in a well-marked maximum in May, and after producing oospores disappearing at the end of May or in early June. Temperature is undoubtedly an important factor, a monthly mean temperature between 6° and 12° C. being most favourable, while growth always took place in this pond with a rising temperature. The exceptional development of the species towards the end of December 1918 (cf. table) is correlated with the fact that this month was abnormally mild, its mean temperature being 6.8° C. (2.9° above normal).

The relation of this alga to the concentration of the water is exceptionally well shown. From a comparison with the data in Fig. 3, it is evident that the development of *Volvox aureus* coincides with the *lowest concentrations recorded during the period of observation*. Information is not available for the April—May maximum of 1918, but growth of the species, whether slight or pronounced, at other times always occurred with a low concentration (falling or rising) between 8 and 14 degrees. The complete absence of *Volvox* from the pond during the dry spring of 1921 was obviously due to the fact that a favourable low concentration was never attained during that season (cf. Fig. 3).

Abundant bright sunshine is not essential for the purely vegetative growth of *V. aureus*, as shown in December 1918 (extremely dull, but very mild), February 1919 (very dull) and April 1920 (rather dull), but it is very different when we consider sexual reproduction in this species. As will be noticed from the frequency-table sexual organs were regularly produced at the end of the spring-maximum (*i.e.* in May) in each of the three complete years, and it has been mentioned several times that May was the sunniest month in each of these years (cf. Fig. 2). Thus abundant sunshine, with a moderate temperature, appears to be an essential condition for

oospore-production in this species (cf. *Spirogyra*, *Mougeotia scalaris*, *Closterium acerosum*, *Cosmarium Botrytis*, *Ædogonium* spp.).

There seems some evidence that abundant aeration of the water may be essential for the growth of *Volvox aureus*, since the various maxima generally come after periods of heavy rainfall, when the pond would receive a fresh supply of dissolved gases from the atmosphere; but it is difficult to disentangle the influence of this factor from that of the low concentration of the water, which undoubtedly is a very favourable condition. Perhaps both factors (low concentration and good aeration) are concerned in the development of the species. It may be noted in this connection that in early May 1920 some puddles of almost pure rain-water (resulting from the heavy rains of April), lying in the marshy region between the pond and the fence (cf. Fig. 1), were bright green in colour owing to an almost pure growth of *Volvox* in them; and the water of these shallow puddles would certainly be better aerated than that of the main body of the pond, where the species was less abundant.

XVIII. THE CYANOPHYCEÆ

The most abundant Blue-green Algæ represented in the pond during the period of observation were *Anabæna oscillarioides*, *Cylindrospermum majus*, *Oscillatoria tenuis*, *O. splendida*, *Phormidium uncinatum*, *P. molle*, *P. tenue* and *Arthrospira Jenneri*. The frequencies of the first two species, and of the species of *Oscillatoria* and *Phormidium* (grouped together and considered as a whole) are given in Fig. 11.

*Anabæna oscillarioides*¹ is a typical summer-form, its period of maximum development being from June to September. The species during these months is abundant not only in the shallower water at the margin of the pond (where it is generally mixed with more or less *Cylindrospermum majus*), but also amongst the filaments of the wide species of *Ædogonium* which predominate in the deeper central parts of the pond during the summer (cf. Section VI). *A. oscillarioides* requires the higher summer-temperatures (a monthly mean between 12° and 16.5° C.) before it becomes common, and it commences development during the first really hot month of the year (generally May or June), its maximum being attained some time during June—August. The persistence of the species into the autumn-months is

¹ Other species of *Anabæna* were possibly present in the pond at times, but were always rare and unimportant.

rather variable (most pronounced in 1919), and is probably determined by a variety of factors, including competition with other species.

With regard to the influence of the concentration of the water the higher concentrations lying between 17 and 27 degrees (cf. data in Fig. 3) are apparently requisite for pronounced growth of *A. oscillarioides*, so that the species is doubtless favoured by abundance of dissolved organic matter in the water (cf. Section IV)—*i.e.* its nutrition is probably mixotrophic. In August 1919, when the very high concentration of 27 degrees was reached, the species was common, but apparently the sudden dilution of the pond which followed in September resulted in adverse conditions for this alga, since it decreased in the latter month (although temperature in September was rather favourable). The adverse influence of a sudden decrease in the concentration (when temperature remains favourable) is also apparent during August—September 1918 and the same months of 1920.

Fritsch and Rich (18) from their observations on *Anabæna catenula* in Barton's pond concluded that this species is "apparently adversely affected by exposure to prolonged summer-sunshine," but there does not seem much evidence to support this in the case of *A. oscillarioides* in Hawkesley Hall pond. In 1918, for instance, the three sunniest months were May, June and July (cf. Fig. 2), and the species had its maximum from the middle of June to the end of July, showing at least that bright sunshine did not appear to affect its development adversely, while the month (August) in which it commenced to decline was rather poor in sunshine. Bright sunshine is certainly not essential for the growth of this species, as shown in 1919 and 1920. The formation of spores in *Anabæna* always followed immediately after any noteworthy development had taken place, and it does not seem to require any special set of conditions apart from those influencing vegetative growth.

Cylindrospermum majus (Fig. 11) is also a summer-form but appears to be adapted to rather lower temperatures than the *Anabæna* since its maximum growth never occurred in the hottest months, but in those with a mean temperature between 12° and 14° C.; while it was best developed in the pond during the cool summer of 1920. It is a form which was undoubtedly adversely affected at times by the growth of *Anabæna*, in company with which it was usually found, and this may explain its curious absence in the summer of 1919. Like *Anabæna* it prefers high or rather high concentrations of the water, but a rapid fall in the concentration (as

in August—September 1920) does not appear to be quite so harmful to *Cylindrospermum* as to *Anabæna*. No relation to bright sunshine is apparent. Spore-formation in this species regularly follows vegetative growth, even when this is very slight (September—October 1919).

All the species of *Oscillatoria* and *Phormidium* which have occurred in the pond during the period of observation exhibit a very similar mode of occurrence, and for that reason they have been grouped together and their frequencies considered as a whole in Fig. 11. As is obvious from the curve these genera are present in quantity only from June to November, although the actual positions of the maxima differ markedly in different years. In each of the three complete years there were two maxima, which in the case of both 1918 and 1919 were due to the development of different species, but in 1920 one of the species (*O. tenuis*) of the first maximum was repeated in the second. Thus in 1918 the first maximum (August) consisted of *O. tenuis* and *O. splendida* (approximately equally developed), while the second maximum (October) consisted almost entirely of *Ph. uncinatum*; in 1919 the first maximum (August) was due to *O. tenuis* (while *O. splendida* was rare), and the second (October—November) was caused by *Ph. tenue* and *O. splendida*, the former being rather the commoner species. The first maximum of 1920 (June—July) was due to *O. tenuis* and *Ph. molle* in equal abundance, while the second (October) was the result of the growth of *O. tenuis*¹ and *O. splendida*, the former being rather better developed than the latter. Two species (*O. tenuis* and *Ph. molle*) were concerned in the rather early development shown in 1921.

A profound relation is shown between the growth of these two genera in the pond and the concentration of the water (cf. data in Fig. 3), and in almost every case the position of each maximum coincides with the attainment of a high concentration. This is naturally most marked in 1919 (the year in which these genera were best developed in the pond) when the very high concentration of August, and the still higher ones of October—November (cf. Fig. 3) exactly correspond with the two maxima of the Oscillatoriaceæ in this year. Of the six chief maxima shown by *Oscillatoria* and *Phormidium* two (August, October—November 1919) coincided with very high concentrations (27 to 30 degrees), two (August 1918, June—July 1920) correspond with moderately high concentrations

¹ Fritsch (14) found that *O. tenuis* in a pond at Telscombe, near Newhaven, had its maximum in October—November, thus agreeing with the present observations.

(18 to 20 degrees), while the two remaining maxima occurred with moderate concentrations. These results are sufficient to show that high concentrations of dissolved organic matter in the water (cf. Section IV) are particularly favourable for the growth of these two genera; in fact one would be fairly safe in predicting that the development of one or more of their species would result from the attainment of a high concentration of the water of this pond during the autumn of any particular year (other conditions being fairly normal).

Temperature is undoubtedly an important factor with these two genera, and high or moderately high temperatures (a monthly mean between 8° and 16° C.) are best suited for their growth. Development almost always commenced during the warmest months of the year although the maxima generally occurred with the temperature falling in the autumn. Temperature appeared to be concerned in the slight local development in January 1921 (an extremely mild month—see Fig. 2), and it was also the cause (together with the favourable concentration-conditions) of the early growth of *O. tenuis* and *Ph. molle* in May 1921. No relation is shown towards bright sunshine.

A few words may be added on the occurrence of *Arthrospira Jenneri*. It showed a slight local development on three occasions, namely August 1919, June—July 1920, and June 1921, and was also noted, but was very rare, during several other months. It appears to be a summer-form, adapted to the higher temperatures, and undoubtedly prefers high concentrations of dissolved organic matter in the water, since on each of the three occasions mentioned above the concentration was high (very high in August 1919—cf. Fig. 3). This species was always observed in company with species of *Oscillatoria* or *Phormidium*, and it seems to be influenced by external conditions much in the same way as these two genera, and like them it also appears to have a mixotrophic mode of nutrition.

XIX. THE FLAGELLATES

Only three species belonging to this group were at all abundant in Hawkesley Hall pond, namely *Cryptomonas ovata*, *Trachelomonas volvocina* and *T. hispida*, and their occurrence is represented in the table on p. 24.

Cryptomonas ovata is a hardy form but generally most abundant in late summer and early autumn (July—September) but although it appears to require summer-temperatures (a monthly mean between 13° and 16.5° C.) for development in any quantity, it may become

Table showing the Occurrence of Three Flagellates in Hawkesley Hall Pond during 1918-1921.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Cryptomonas ovala</i>	1918	vr	vr	vr	r	rr	rr	rr	rc	rr	rr	r	r
	1919	vr	vr	vr	r	vr	vr	rr	rr	rr	r	rr	rr
	1920	vr	vr	vr	r	r	r	r	rc	rr	vr	vr	vr
	1921	vr	vr	vr	r	r	r						
<i>Trachelomonas volvocina</i>	1918	vr	vr	r	r	rc	rc	r	r	r	r	r	vr
	1919	vr	vr	r	rr	rr	r	vr	rr	vr	vr	vr	vr
	1920	vr	vr	r	rr	rr	rc	rr	rc	rr	r	vr	vr
	1921	vr	vr	vr	vr	r	r						
<i>Trachelomonas hispida</i>	1918	—	—	vr	vr	r	rr	—	—	—	—	—	vr
	1919	—	—	—	r	rc	r	vr	vr	—	—	—	vr
	1920	vr	vr	vr	rr	r	r	vr	rr	rr	vr	—	—
	1921	—	—	—	vr	vr	r						

“rather rare” without showing much relation to temperature (cf. its slight development November—December 1919). The species was always poorly represented during the winter and early spring. High or rather high concentrations of the water are decidedly favourable, rapid growth always coinciding with a concentration between 17 and 21 degrees, and still higher concentrations (August and November 1919—cf. Fig. 3) are far less unfavourable than lower ones, in fact low concentrations, especially when combined with low temperatures, are obviously very antagonistic towards the growth of this species. No relation to bright sunshine is apparent.

Trachelomonas volvocina (cf. table above) exhibits a rather puzzling periodicity, which is not closely related to temperature-conditions, although the lower temperatures of winter are clearly unfavourable. Growth commences earlier in the year than with *Cryptomonas*, and the maximum is also reached earlier (often in spring), and sometimes there is a secondary maximum in August (1919, 1920), while the species is generally rare during the autumn¹. Its occurrence in the pond appears to be best explained by reference to bright sunshine (Fig. 2) and the concentration-data (Fig. 3). Of the five maxima observed, three (May—June 1918, August 1919, June 1920) occurred in very sunny months, and also when the

¹ Kofoid (22) found that *T. volvocina* was best developed in the plankton during the warmer summer months. On the other hand Fritsch and Rich (18) found that this species had its maxima frequently at the end of winter or during early spring, although at other times the maximum of *T. volvocina* and *T. hispida* coincided with the warmer months of the year.

concentration was high (18 to 27 degrees), one (April—May 1919) took place with a low concentration (rising to 18 degrees by the end of May), while the fifth (August 1920) coincided with a rather dull month, although the concentration was moderately high (18 degrees). Thus although the occurrence of *T. volvocina* cannot be explained by either bright sunshine or the concentration alone, the conjunction of abundant sunshine with a high (or rather high) concentration of the water, and during the warmer months of the year, appears to supply the best conditions for growth. Probably some other factor (perhaps competition) has controlled the occurrence of this species in the pond.

It may be suggested that *T. volvocina* can to some extent vary its mode of nutrition in nature, being at times mainly (or wholly) autotrophic, when supplied with abundant bright sunshine¹, and without any considerable amount of organic matter in the water, while at other times, when supplied with large quantities of organic matter, it may have a mixotrophic method of nutrition and thus do away with the necessity for bright sunlight. This tentative suggestion would perhaps best explain the occurrence of the species in the present pond. Naumann² has suggested that the probable cause of the abundant development of *T. volvocina*, forming a chocolate-coloured water-bloom in a tank in the Botanic Gardens at Lund, was the supply of organic matter in the water; in the case of Hawkesley Hall pond, however, this factor—although probably an important one—cannot completely explain the occurrence of the species.

Trachelomonas hispida was generally less abundant in the pond than the preceding species, but its periodicity (cf. table above) does not differ in any important respect from that of *T. volvocina*. None of its "maxima," however, falls with a concentration of the water above 18 degrees, and the slight development in April 1920 occurred with an extremely low concentration, so that perhaps this species may not be able to utilise organic matter in the water to the same extent as *T. volvocina*. The two maxima of *T. hispida* in 1918 and 1919 coincide with periods of very abundant bright sunshine, which is further evidence that the species is mainly autotrophic in its mode of nutrition.

¹ The translucent brown shell would explain the necessity for this condition if the species is to any extent autotrophic.

² *Bot. Notiser*, Lund, 1913, p. 249, etc.

XX. GENERAL CONCLUSIONS REGARDING THE CONCENTRATION OF THE WATER

Up to the present very little *exact* work has been done by algologists on the relation between the growth of the various algal species in nature and the concentration of the water, although several workers on algal periodicity have dealt with the influence of this factor in a more or less speculative manner. Fritsch and Rich (14, 16) were perhaps the first to point out that the concentration of the water was an important factor concerned in the growth of freshwater algæ in nature. In the present study, as was indicated in the Introduction, special attention was directed towards concentration-conditions, and although the methods used in this connection (see Section IV) are not altogether above criticism from the chemical standpoint, yet the results obtained show conclusively that with many algæ the concentration of the water is a very important factor controlling their occurrence in nature. On the one extreme we get forms like *Volvox aureus* (see Section XVII) adapted to the very lowest concentrations observed in the pond, and on the other there are several species (*Tribonema affine*, *Chlamydomonas Reinhardi*, *Oscillatoria* spp., *Phormidium* spp.) the occurrence of which to a great extent is determined by very high concentrations, while each concentration observed during the period of observation has at least one (usually several) algal species, the development of which it tends to favour, although of course it is always possible for other factors to step in and influence growth in some way.

The origin of the high concentrations observed at certain times in Hawkesley Hall pond has been discussed in Section IV, where it was shown that the marked fluctuation noted in the concentration was due not only to the effects of evaporation, and dilution by rains, but also to the addition of soluble organic matter to the water by the decay during certain months of the year of large quantities of the floating leaves of *Potamogeton natans*, as well as masses of various filamentous algæ. It became apparent during the course of the work that a correct idea of the concentration could not be obtained from a record of the water-level of the pond, and still less from an examination of the rainfall-data. It was also shown that soluble organic matter made up the greater part (0.6 to 0.8) of the total soluble matter in the water, so that there is reason to believe that those species which are favoured by high concentrations are probably more or less mixotrophic in their mode of nutrition in nature.

It has been shown by certain workers that many algæ in cultures prefer a mixotrophic method of nutrition, and there is no doubt that further research on the lines suggested by the present paper will show that the same obtains with many algal species (especially certain of the Volvocales¹, and many Blue-green Algæ, and also Flagellates) in nature, more particularly those which at times develop in great abundance in the form of "water-bloom²."

XXI. SUMMARY OF THE CHIEF RESULTS

Spirogyra (Section VII) is best represented in the spring-phase (maximum in May) although *S. inflata*, *S. protecta*, *S. colligata* and *S. varians* appeared and conjugated in the autumnal phase (October—November). A monthly mean temperature between 6° and 12° C. appears to be best for the growth of this genus, although the hardier species (*S. inflata*, *S. protecta*) can tolerate much lower temperatures. *S. bellis* appears to prefer rather higher temperatures. Abundant bright sunshine is very essential for growth of the more sensitive species of *Spirogyra*, but *S. inflata* and *S. protecta* may develop in months rather poor in sunshine. A moderately low concentration of the water between 12 and 18 degrees in the pond is best suited for *Spirogyra*, although again *S. inflata* and *S. protecta* are more tolerant in this connection than the other more sensitive species (*S. catenæformis*, *Grevilleana*, *condensata*, *Jurgensii*, *mirabilis*, *longata*, *maxima*, *Weberi*); very high concentrations are particularly harmful, and prevented the development of an autumnal phase of *Spirogyra* in 1919. A sudden increase in the concentration is unfavourable towards the more sensitive species of the genus.

Zygnema (Section VIII) has its maximum at the end of April or in early May, but may be rather common again in the autumn. It is very hardy but became really abundant only when the temperature was moderate (a monthly mean between 5° and 12° C.), and the concentration of the water low or moderately low (10 to 17 degrees).

Mougeotia scalaris (Section VIII) is a hardy form as regards its ability to withstand extremes of temperature, and varying concentrations of the water. Abundant bright sunshine is not necessary for good vegetative growth, but is very essential for zygospore-

¹ Although certainly not in the case of *Volvox*, the highest member of this order (cf. Section XVII).

² Cf. in this connection the observations of Plümecke on *Gonium pectorale* (in *Ber. Deutsch. Bot. Ges.* 32, 1914, pp. 131–136); and also the paper of Kolkwitz and Marsson (23).

production. The form was much influenced by competition with other filamentous Conjugates in the pond. In the case of *M. viridis* abundant bright sunlight does not seem to be so essential for conjugation as with *M. scalaris*, and it is also adapted to lower temperatures.

The Desmids of the pond (Section IX), as regards the position of their maxima, tend to fall into two groups, (1) those which have their maximum in spring or autumn (*Hyalotheca dissiliens*, *Sphaerosma granulatum*, *Closterium acerosum*), and (2) those which attain a maximum in summer or early autumn (*Closterium moniliferum*, *Cl. Kützingii*, *Cl. lineatum*, *Pleurotænium Ehrenbergianum*, *Cosmarium Botrytis*, *Staurastrum brevispinum*, *S. Dickiei*, and others). *Closterium striolatum* tends to connect the two groups. Those of the first group prefer moderate temperatures (usually a monthly mean between 6° and 13° C.) and rather moderate concentrations (between 10 and 18 degrees); those of the second group are adapted to higher temperatures (a monthly mean between 10° and 16° C.), and somewhat higher concentrations (between 14 and 21 degrees, although differing slightly with the different species).

The three species of *Staurastrum* were more tolerant of higher concentrations (up to 27 degrees) than the *Cosmarium* spp. The Desmids of the pond which have conjugated fall into two classes, (1) those which require abundant bright sunshine before conjugation can take place (*Closterium acerosum*, *Cosmarium Botrytis*), and (2) those which are able to conjugate given only a moderate amount of sunshine (*Staurastrum brevispinum*, *S. Dickiei*, *Closterium lineatum*, *Cl. rostratum*).

The species of *Ædogonium* (Section X) present in the pond fall into two groups; (1) includes a wide sterile species which attains a great maximum during June—August, and (2) narrower species (*O. cryptoporum*, *O. crispum*, *O. Borisianum*, *O. Braunii*, *O. echinospermum*, and *O. rugulosum*) which were at a maximum in May (or early June), and rapidly declined in June after fruiting. The wide sterile species of the first group is favoured by the higher summer-temperatures (a monthly mean between 12° and 16.5° C.), and no relation is shown towards bright sunshine, and little to the concentration of the water. The narrower species of group 2 are typical spring-forms favoured by a monthly mean temperature between 10° and 13.5°, the higher temperatures being unfavourable; and their maxima always occurred after a period of very sunny weather, which supports the view that abundant sunshine is essential for oospore-

production in these species; while medium concentrations of the water, between 16 and 20 degrees, are most favourable. *Bulbochæte minor* closely resembles the narrower species of *Edogonium* as regards the conditions requisite for its development and reproduction.

Tribonema bombycinum (Section XI) develops best with a falling temperature in autumn, and remains abundant during the lower temperatures of winter. It is very adversely affected by bright sunshine, and tends to develop in parts of the pond shaded by the leaves of various phanerogamic plants. It is very tolerant of almost all degrees of concentration of the water, the very highest concentrations being only slightly unfavourable; but a very abrupt fall in the concentration may be harmful. *T. affine* agrees closely with the former species in the matter of temperature and also sunshine, but prefers the very highest concentrations observed during the period of observation, and thus is probably mixotrophic in its mode of nutrition in nature.

Microspora floccosa (Section XII) tends to develop during the colder months (with a mean monthly temperature between 1.5° and 5.5° C.); and bright sunshine is not essential for its growth, and is probably harmful. Little or no relation is shown towards the varying concentration of the water, probably because competition with *Tribonema* tended to greatly limit its growth in the pond. *M. stagnorum* is also a cold-water species which was never abundant in the pond owing to competition with *Tribonema* and *M. floccosa*.

It is a general rule that the occurrence of any alga which is struggling to develop under conditions in which it is adversely influenced by competition with other species (of animals or plants) will tend to show little relation to the various meteorological conditions or to the concentration of the water, since *competition* is the particular factor which sets a limit to the growth of the species, even though all other factors may be favourable towards its increased growth.

Vaucheria aversa (Section XIII) prefers a low or moderately low temperature (a monthly mean between 4.5° and 8° C.), abundant aeration of the water, and probably also a low or rather low concentration of the latter; while bright sunshine is not at all an essential factor for oospore-production. *V. sessilis* apparently differs in its requirements from *V. aversa*, but the evidence is incomplete.

Aphanochæte repens (Section XIV) tends to be a perennial species in the pond, regularly showing a maximum in spring, and sometimes a secondary maximum in autumn. It prefers moderate temperatures

(a monthly mean between 4° and 11° C.), higher summer-temperatures being very unfavourable. Low or moderately low concentrations of the water (10 to 17 degrees) must obtain for growth to take place; and its development is conditioned by the presence of a suitable quantity of the "host" (the wide sterile species of *Edogonium*).

Nitella flexilis (Section XV) apparently develops most rapidly when the temperature is moderately high (a monthly mean between 9° and 14° C.); and abundant bright sunshine, although not essential for purely vegetative growth, is very necessary for oospore-production. No relation to the concentration of the water is shown. The growth of the endophytic *Coleochæte Nitellarum* was always limited by the somewhat local growth of its "host," but abundant sunshine is essential for the production of the sexual organs of this endophyte.

The various species of the Protococcales (Section XVI) which have been at all abundant in the pond form a sort of series as regards the temperature most favourable for their development. On the one hand *Ankistrodesmus falcatus* requires a monthly mean temperature between 11° and 14° C., while the higher summer-temperatures appear to be unfavourable, and on the other hand *Pediastrum Boryanum* and *P. tetras* are adapted to the highest summer-temperatures, while *Scenedesmus denticulatus* var. *linearis* and *Dictyosphaerium* are intermediate as regards their behaviour towards temperature. All (except *Pediastrum*) seem to develop best with moderately high concentrations between 16 and 20 degrees. *Pediastrum* shows no relation to the concentration of the water.

Chlamydomonas Reinhardi (Section XVII) tends to develop best in the pond during late summer and early autumn (September), when the temperature is falling (monthly mean temperature between 16.5° and 13° C.). A high or rather high concentration (18 to 27 degrees) favours growth, and the species is probably mixotrophic in its nutrition in nature. *Chl. Debaryana* and *Chl. monadina* are also apparently favoured by the presence of organic compounds in the water, but are adapted to lower temperatures than *Chl. Reinhardi*.

Pandorina and *Eudorina* (Section XVII) both tend to have their periods of growth during the summer-months (May—August) although *Eudorina* generally persisted in some quantity well into the autumn, and was generally rather more hardy than *Pandorina*. The behaviour of both species during the summer-months was very variable and was probably the result of mutual competition, and perhaps also competition with other algæ.

Volvox aureus (Section XVII) is a spring-form with a maximum usually in May. Moderate temperatures (a monthly mean temperature between 6° and 12° C.) and a low or very low concentration of the water are essential conditions for development. Abundant bright sunshine is not at all necessary for purely vegetative growth, but is very essential for oospore-production.

Anabæna oscillarioides (Section XVIII) is a summer-form favoured by the higher temperatures (a monthly mean between 12° and 16.5° C.) and also by high or very high (17 to 27 degrees) concentrations of the water. Its nutrition is probably mixotrophic. A sudden decrease in the concentration is very unfavourable for this species. Little or no relation towards bright sunshine is shown. *Cylindrospermum majus* is also a summer-form adapted to rather lower temperatures than *Anabæna*, and prefers a monthly mean temperature between 12° and 14° C. It is adapted to high or rather high concentrations, but its growth in the pond was adversely affected by *Anabæna*.

Oscillatoria, *Phormidium* and *Arthrospira Jenneri* (Section XVIII) show a marked relation to the concentration of the water, the position of a maximum generally coinciding with a high concentration, so that these algæ are doubtless mixotrophic. High or moderately high temperatures (a monthly mean between 8° and 16° C.) appear to be best, the maxima usually occurring in the autumn. No relation to bright sunshine is apparent.

Cryptomonas ovata (Section XIX) is a hardy form, but generally more abundant during July—September (and when the monthly mean temperature is between 13° and 16.5° C.). High or rather high concentrations (between 17 and 21 degrees) are decidedly favourable; while no relation is shown towards bright sunshine.

Trachelomonas volvocina (Section XIX) shows a periodicity which appears to be best explained by reference to bright sunshine and the concentration-conditions, since the conjunction of abundant sunshine with a high (or rather high) concentration, and during the warmer months of the year, appears to supply the best conditions for growth. It is suggested that this species may to some extent be able to vary its mode of nutrition in nature, being under certain conditions mainly autotrophic and under other circumstances mixotrophic. *T. hispida* agrees closely in its mode of occurrence with *T. volvocina* but its development always occurred with moderate concentrations.

In conclusion I wish to acknowledge my indebtedness to the late

Professor G. S. West for his very kind interest in the early part of this work, and for his help in identifying several of the algal species (particularly the Desmids) which have occurred in the pond.

BIBLIOGRAPHY

- (1) ANDERSEN, E. N. and WALKER, E. R. An Ecological Study of the Algæ of some Sandhill Lakes. *Trans. Amer. Micros. Soc.* **39**, No. 1 (January), 1920, pp. 51-85.
- (2) BENECKE, W. Ueber die Ursachen der Periodicität im Auftreten der Algen, auf Grund von Versuchen über die Bedingungen der Zygotenbildung bei *Spirogyra communis*. *Internat. Rev. d. Gesamt. Hydrobiologie u. Hydrogr.* **1**, 1908, pp. 533-552.
- (3) BIRGE, E. A. and JUDAY, C. The Inland Lakes of Wisconsin. The Dissolved Gases of the Water and their Biological Significance. *Bull. Wisconsin Geol. and Nat. Hist. Survey*, **22**, 1911.
- (4) BROWN, H. B. Algal Periodicity in certain Ponds and Streams. *Bull. Torrey Bot. Club*, **35**, 1908, pp. 223-248.
- (5) CHAMBERS, C. O. The Relation of Algæ to Dissolved Oxygen and Carbon-dioxide, with Special Reference to Carbonates. *Rep. Missouri Bot. Gard.* **23**, 1912, pp. 171-207.
- (6) COMÈRE, J. Observations sur la périodicité du développement de la flore algologique dans la région toulousaine. *Bull. Soc. Bot. France*, **53**, 1906, pp. 390-407.
- (7) — De l'évolution périodique des Algues d'eau douce dans les formations passagères. *Bull. Soc. Bot. France*, **57**, 1910, pp. 558-563.
- (8) — De l'action du milieu considérée dans ses rapports avec la distribution générale des algues d'eau douce. *Mém.* **25**, *Bull. Soc. Bot. France*, **16**, 1913, pp. 1-96.
- (9) COPELAND, W. F. Periodicity in *Spirogyra*. *Bot. Gaz.* **47**, 1909, pp. 9-25.
- (10) DANFORTH, C. H. Periodicity in *Spirogyra*, with special reference to the work of Benecke. *Rep. Missouri Bot. Gard.* **21**, 1910, pp. 49-59.
- (11) DÉGLON, A. Contribution à la flore paludéenne des environs d'Yverdon. *Bull. Soc. Vaudoise d. Sci. Nat.* **53**, No. 198, 1920, pp. 23-75.
- (12) DELF, E. M. The Algal Vegetation of some Ponds on Hampstead Heath. *New Phytol.* **14**, 1915, pp. 63-80.
- (13) FRITSCH, F. E. Algological Notes, IV. Remarks on the Periodical Development of the Algæ in the Artificial Waters at Kew. *Ann. Bot.* **17**, 1903, pp. 274-278.
- (14) — Problems in Aquatic Biology, with special reference to the Study of Algal Periodicity. *New Phytol.* **5**, 1906, pp. 149-169.
- (15) — The Subaerial and Freshwater Algal Flora of the Tropics. *Ann. Bot.* **21**, 1907, pp. 235-275.
- (16) FRITSCH, F. E. and RICH, F. Studies on the Occurrence and Reproduction of British Freshwater Algæ in Nature, I. Preliminary Observations on *Spirogyra*. *Ann. Bot.* **21**, 1907, pp. 423-436.
- (17) — *Ibid.*, II. A Five Years' Observation of the Fish Pond, Abbot's Leigh, near Bristol. *Proc. Bristol Nat. Soc.* 4th ser., **2**, 1909, pp. 27-54.
- (18) — *Ibid.*, III. A Four Years' Observation of a Freshwater Pond. *Ann. d. Biologie lacustre*, **6**, 1913.
- (19) GRIFFITHS, B. M. The Algæ of Stanklin Pool, Worcestershire; an Account of their Distribution and Periodicity. *Proc. Birmingham Nat. Hist. and Phil. Soc.* **12**, 1912, No. 5.
- (20) HODGETTS, W. J. A New Species of *Spirogyra* (*S. colligata*). *Ann. Bot.* **34**, 1920, pp. 519-524.

- (21) KLEBS, G. *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*. Jena, 1896.
- (22) KOFOID, C. A. The Plankton of the Illinois River. Part II. Constituent Organisms and their Seasonal Distribution. *Bull. Ill. State Lab. Nat. Hist.* **8**, 1908, Article 1.
- (23) KOLKWITZ, R. and MARSSON, M. Ökologie der pflanzlichen Saprobien. *Ber. Deutsch. Bot. Ges.* **26A**, 1908, pp. 505-519.
- (24) PEVALEK, I. O biologiji i o geografskom rasprostranjenju algâ u sjevernoj Hrvatskoj.—Zur Kenntniss der Biologie und der geographischen Verbreitung der Algen in Nordkroatien. *Izvješća o raspravama matem.-prir. razr.* (*Bull. d. Travaux de la Classe d. Sci. Mat. et Nat.*). Zagreb, **5**, 1916.
- (25) PLATT, E. L. The Population of the "Blanket-Algæ" of Freshwater Pools. *Amer. Nat.* **49**, 1915, pp. 752-762.
- (26) RABANUS, A. Beiträge zur Kenntnis der Periodizität und der geographischen Verbreitung der Algen Badens. *Ber. der Naturforsch. Ges. zu Freiburg i. Br.* **21**, 1915.
- (27) TRANSEAU, E. N. The Periodicity of Algæ in Illinois. *Trans. Amer. Microscop. Soc.* **32**, 1913, pp. 31-40.
- (28) ——— The Periodicity of Freshwater Algæ. *Amer. Journ. Bot.* **3**, 1916, pp. 121-133.
- (29) WALKER, N. Pond Vegetation. *The Naturalist*, Oct. 1905 (No. 585), pp. 305-311.
- (30) WEST, G. S. The Algæ of the Yan Yean Reservoir, Victoria: A Biological and Ecological Study. *Journ. Linn. Soc. London, Bot.* **39**, 1919, pp. 1-88.
- (31) ——— *Algæ*, **1**, 1916, Cambridge.
- (32) WEST, W. and WEST, G. S. The Phytoplankton of the English Lake District. *The Naturalist*, March—Sept. 1909.
- (33) ——— The British Freshwater Phytoplankton, with special reference to the Desmid-Plankton and the Distribution of British Desmids. *Proc. Roy. Soc. B*, **81**, 1909, pp. 165-206.
- (34) ——— On the Periodicity of the Phytoplankton of some British Lakes. *Journ. Linn. Soc. London, Bot.* **40**, 1912, pp. 395-432.
- (35) WHIPPLE, G. C. and PARKER, H. N. On the Amount of Oxygen and Carbonic acid in Natural Waters and the Effects of these Gases upon the Occurrence of Microscopic Organisms. *Trans. Amer. Microscop. Soc.* **23**, 1902, pp. 103-144.

STUDIES IN PHÆNOLOGY, No. 3, 1921¹

By FRANCIS DARWIN

THE most obvious character of the dates of flowering in 1921 is their remarkable earliness. My phænological work was begun in 1917, so that I have only the record of four years with which to compare the facts of 1921. Of these four years 1918 obviously shows the earliest dates of flowering. I have therefore compared 1921 with 1918. And where I have recorded (in 1921) the flowering dates of species not occurring in 1918, I have compared them with the mean dates given by Blomefield².

In *Nature*, Sept. 15, 1921, p. 96, a useful summary of the Meteorological conditions for 1921 is given. It appears that: "The mean temperature was above the normal in all districts with the exception of north and east Scotland, the greatest excess being 23° F. in the Midland counties³."

The following table shows the deviation from normal at Clifton:

Week no.	Date	Deviation from normal	Week no.	Date	Deviation from normal
1	Jan. 1-8	+6.5	21	May 22-28	+4.3
2	9-15	+4.4	22	29-Ju. 4	-1.9
3	16-22	+6.0	23	Ju. 5-11	+1.0
4	23-29	+8.0	24	12-18	+3.1
5	30-Feb. 5	-0.8	25	19-25	+2.9
6	Feb. 6-12	-2.1	26	26-July 2	+0.3
7	13-19	+2.8	27	July 3-9	+3.6
8	20-26	+3.7	28	10-16	+8.2
9	27-Mar. 5	+2.0	29	17-23	+6.8
10	Mar. 6-12	+1.7	30	24-30	+2.4
11	13-19	+1.8	31	31-Aug. 6	+0.6
12	20-26	+5.6	32	Aug. 7-13	-4.3
13	27-Apr. 2	+2.6	33	14-20	+0.2
14	Apr. 3-9	+1.2	34	21-27	+0.6
15	10-16	-0.1	35	28-Sep. 3	-2.9
16	17-23	-3.9	36	Sep. 4-10	+2.8
17	24-30	+2.3	37	11-17	-1.5
18	May 1-7	-1.9	38	18-24	+1.2
19	8-14	+1.8	39	25-Oct. 1	-0.2
20	15-21	-0.5			

¹ Nos. 1 and 2 were published in the *New Phytologist*, 18 and 20.

² In his *Naturalist's Calendar*. Cambridge, at the University Press, 1903.

³ Sir Napier Shaw (*The Times*, Oct. 15, 1921) writes: "The weather of the British Isles...has been remarkable for its drought, its abundant sunshine and its exceptional warmth."

In regard to other conditions it appears that "the rainfall was decidedly less than normal except in west Scotland." Indeed the summer deficiency in rainfall is 5.42 inches, and "the total is only 19 per cent. of the average." The character of the rainfall is strikingly expressed as being "the smallest in the last 105 years." It is interesting to find that the next lowest rainfall occurred in 1918 when the flowering dates were decidedly early but not as strikingly so as in 1921.

It is not surprising that, with a temperature above normal, the flowering dates of 1921 should be early. The connexion of low rainfall with early flowering is probably to be ascribed partly to the quicker warming up of the drier soil in the spring, so that early *growth* would be promoted through the higher soil temperature, provided sufficient water is present for the needs of growth. In regard to early flowering, as such, it is well known that individual plants growing in spots where the supply of water is soon exhausted produce a smaller bulk of vegetative organs and flower correspondingly earlier, though of course less profusely, than those growing in spots where water is available for a longer period. This can be seen very clearly by comparing the individuals of *Draba verna*, *Cerastium*, *Myosotis*, etc., growing on the very thin soil on an old wall with those growing on deeper soil. By flowering early such plants seize the opportunity of setting some seed, however little, before the thin soil on which they grow has quite dried up. This state of things applies primarily to annuals, but if relative lack of water (and mineral salts) has a direct effect not only in checking vegetative growth, but in promoting flowering as such, we should expect to find it tending to produce early flowering in perennials also.

When we analyse the flowering dates for 1921¹ we find that from January 1 to July 1, *i.e.* in the first 198 flowering-dates, as nearly as possible three-fifths of the dates are "early." From July 3 to August 6 the dates are almost all "late," but since in this period a preponderating number cannot be described as either late or early (from want of observations with which to compare them) we cannot include them in the inquiry. From August 6 to September 16, the late flowerings decidedly preponderate, but the total number in this period is small, viz. 13 in number.

If we include the whole number of dates, viz. 264 in number, we find that 0.61 are early.

¹ When the name of a plant is preceded by the letter C, such a species was found near Cambridge. In the same way S means that the species was noted in Surrey (near Gomshall). The remainder, whose names are not marked either C or S, were found at Brookthorpe near Gloucester. One plant, *Colchicum autumnale*, was found near Hereford.

PHÆNOLOGICAL TABLES: Order of Flowering, 1921

No.	1920 (end of)	Name	No.	1921	Name
C 1	Nov. 14 ¹	Taraxacum Dens-leonis	52	Apr. 15	Ranunculus aquatilis
C 2	17 ¹	Primula veris	53	15	Alopecurus pratensis
C 3	17 ¹	Bellis perennis	54	15	Chelidonium majus
C 4	19 ¹	Petasites fragrans	55	16	Carduus crispus
			56	17	Viola tricolor
	1921		57	23	Ajuga reptans
5	Jan. 3	Corylus Avellana	58	24	Viburnum lantana
6	7	Galanthus nivalis (garden)	59	25	Carex pendula
7	10	Ranunculus Ficaria	60	26	Anthoxanthum odoratum
8	11	Lamium album	61	26	Medicago lupulina
9	24	Mercurialis perennis	62	28	Acer Pseudo-platanus
10	25	Ranunculus repens	63	28	Trifolium pratense
11	27	Chærophyllum sylvestre	64	29	Veronica montana
12	Feb. 1	Fragaria vesca	65	29	Geranium phœum
13	8	Ulex europæus	66	30	Cratægus monogyna
14	8	Vinca minor	67	30	Poterium sanguisorba
15	12	Ulmus montana	68	30	Ranunculus acris
16	13	Alnus glutinosa	69	May 1	Ranunculus bulbosus
17	14	Caltha palustris	70	1	Geum urbanum
18	14	Cardamine pratensis	71	2	Sanicula europæa
19	16	Tussilago Farfara	72	3	Ajuga reptans (pink form)
20	16	Adoxa Moschatellina	73	4	Orchis Morio
21	17	Veronica agrestis	74	4	Potentilla Tormentilla
22	17	Lamium purpureum	75	5	Potentilla anserina
23	20	Capsella Bursa-pastoris	76	6	Rubus cæsius
24	22	Lamium maculatum	77	6	Scrophularia nodosa
25	23	Viola sylvatica	78	6	Vicia sativa
C 26	Mar. 6	Stellaria media	79	6	Geranium dissectum
27	6	Viola odorata	80	6	Veronica serpyllifolia
28	6	Anemone nemorosa	81	7	Asperula odorata
C 29	17	Nepeta Glechoma	82	8	Stachys sylvatica
C 30	20	Prunus spinosa	83	8	Sisymbrium officinale
C 31	22	Poa annua	84	9	Carex sylvatica
C 32	22	Veronica hederæfolia	85	10	Lychnis Flos-cuculi
C 33	26	Fraxinus excelsior ♂	86	10	Nasturtium officinale
C 34	28	Heracleum Sphondylium	87	12	Bunium flexuosum
C 35	Apr. 1	Alliaria officinalis	88	12	Veronica Beccabunga
C 36	4	Pyrus Aucuparia	89	13	Ranunculus sceleratus
C 37	4?	Sambucus nigra	90	13	Trifolium incarnatum
38	9	Lamium Galeobdolon	91	14	Lotus corniculatus
39	9	Stellaria Holostea	92	14	Melica uniflora
40	9	Plantago lanceolata	93	15	Tainus communis
41	9	Arum maculatum	94	15	Rhinanthus Crista-galli
42	9	Cardamine hirsuta	95	17	Galium Aparine
43	10	Geranium Robertianum	96	17	Ranunculus arvensis
44	10	Paris quadrifolia	97	17	Rumex Acetosa
45	10	Ranunculus auricomus	98	18	Rosa canina
		var. depauperatus	99	18	Hypochaeris radicata
46	11	Veronica Chamædrys	100	19	Chrysanthemum leucanthemum
47	11	Pedicularis sylvatica			
48	12	Linaria Cymbalaria	101	20	Ægopodium Podagraria
49	13	Allium ursinum	102	21	Orchis maculata
50	13	Vicia sepium	103	21	Carduus palustris
51	14	Lychnis diurna	104	22	Urtica dioica (? sex)

¹ = Jan. 1, 1921.

PHÆNOLOGICAL TABLES—continued

No.	1921	Name	No.	1921	Name
105	May 23	Bryonia dioica ♀ ¹	159	Ju. 15	Geranium pratense
106	23	Plantago media	160	15	Centaurea Scabiosa
107	23	Hieracium Pilosella	161	15	Galium mollugo
108	25	Oenanthe pimpinelloides	162	16	Genista tinctoria
109	25	Bromus arvensis	163	16	Malva sylvestris
110	25	Milium effusum	164	17	Verbascum Thapsus
111	27	Tragopogon pratensis	165	17	Potentilla reptans
112	27	Fumaria officinalis	166	17	Hypericum hirsutum
113	28	Sonchus arvensis	167	17	Arrhenatherum avenaceum
114	28	Epilobium montanum	168	18	Chlora perfoliata
115	28	Solanum dulcamara	169	18	Circaea lutetiana
116	29	Crepis taraxacifolia	170	18	Lactuca muralis
117	30	Linum catharticum	171	18	Rubus fruticosus
118	30	Trifolium repens	172	18	Lapsana communis
119	30	Onobrychis sativa	173	19	Carduus acanthoides
120	30	Scabiosa arvensis	174	19	Pastinaca sativa
121	30	Avena pratensis	175	19	Scabiosa columbaria
122	Ju. 1	Cynosurus cristatus	176	20	Carduus arvensis ♀
123	1	Euphrasia officinalis	177	20	Convolvulus arvensis
124	1	Parietaria officinalis	178	21	Vicia cracca
125	2	Dactylis glomerata	179	21	Plantago major
126	3	Pyrola minor	180	22	Eupatorium cannabinum
127	3	Helianthemum vulgare	181	22	Campanula Trachelium
128	3	Thymus serpyllum	182	22	Epilobium parviflorum
129	3	Hieracium murorum	183	23	Agrimonia Eupatorium
130	3	Roseda luteola	184	23	Galium verum
131	3	Holcus lanatus	185	24	Apium nodiflorum
132	5	Spiraea ulmaria	186	24	Hordeum pratense
133	5	Ophrys apifera	187	25	Malva moschata
134	5	Bryonia dioica ♂	188	25	Ononis arvensis
135	5	Anthyllis vulneraria	189	25	Avena flavescens
136	5	Papaver dubium	190	26	Epilobium hirsutum
137	5	Lolium perenne	191	26	Phleum pratense
138	5	Ophrys muscifera	192	26	Æthusa Cynapium
139	6	Cephalanthera grandiflora	193	27	Asperula cynanchica
140	6	Listera ovata	194	27	Carduus acaulis
141	6	Orchis pyramidalis	195	27	Sedum acre
142	8	Scrophularia aquatica	196	27	Sedum album
143	8	Anagallis arvensis	197	28	Bromus asper
144	8	Ligustrum vulgare	198	28	Triticum caninum
145	8	Silaus pratensis	199	29	Ononis spinosa
146	8	Briza media	200	29	Anthemis Cotula
147	8	Cornus sanguinea	201	30	Lychnis Githago
148	8	Oenanthe pimpinelloides	202	30	Bartsia Odontites
149	8	Lathyrus pratensis	203	30	Achillea Millefolium
150	9	Matricaria Chamomilla	204	30	Calamintha Clinopodium
151	11	Centaurea nigra	205	July 1	Stachys Betonica
152	12	Epilobium angustifolium	206	3	Convolvulus sepium
153	12	Senecio Jacobæa	C 207	7	Melilotus officinalis
154	12	Daucus Carota	C 208	11	Humulus lupulus
155	13	Lysimachia nummularia	C 209	20	Dipsacus sylvestris
156	13	Convolvulus arvensis	S 210	20	Teucrium Scorodonia
157	13	Ballota nigra	S 211	23	Lycopsis arvensis
158	14	Poa pratensis	S 212	26	Erodium cicutarium

¹ The comparison is with a female plant in 1918. The male plant was late

PHÆNOLOGICAL TABLES—*continued*

No.	1921	Name	No.	1921	Name
S 213	July 28	<i>Lychnis vespertina</i>	S 234	Aug. 3	<i>Lonicera Periclymenum</i>
S 214	29	<i>Anthemis Cotula</i>	S 235	4	<i>Claytonia perfoliata</i>
S 215	30	<i>Artemisia vulgaris</i>	S 236	5	<i>Caucalis infesta</i>
S 216	30	<i>Campanula rotundifolia</i>	S 237	6	<i>Bartsia Odontites</i>
S 217	30	<i>Anthemis arvensis</i>	S 238	6	<i>Inula Conyza</i>
S 218	31	<i>Pedicularis palustris</i>	S 239	6	<i>Mentha aquatica</i>
S 219	31	<i>Rumex acetosella</i>	S 240	6	<i>Clematis vitalba</i>
S 220	31	<i>Erica cinerea</i>	S 241	6	<i>Origanum vulgare</i>
S 221	31	<i>Calluna vulgaris</i>	S 242	8	<i>Poa aquatica</i>
S 222	31	<i>Senecio sylvaticus</i>	S 243	10	<i>Sedum Telephium</i>
S 223	Aug. 1	<i>Solidago Virga-aurea</i>	S 244	11	<i>Sparganium racemosum</i>
S 224	1	<i>Arctium Lappa</i>	S 245	11	<i>Lycopus europæus</i>
S 225	1	<i>Angelica sylvestris</i>	S 246	12	<i>Polygonum Persicaria</i>
S 226	1	<i>Solanum nigrum</i>	S 247	15	<i>Verbascum nigrum</i>
S 227	1	<i>Pimpinella saxifraga</i>	S 248	16	<i>Jasione montana</i>
S 228	1	<i>Linaria vulgaris</i>	S 249	17	<i>Polygonum convolvulus</i>
S 229	1	<i>Hypericum perforatum</i>	250	20	<i>Senecio erucifolius</i>
S 230	2	<i>Silene inflata</i>	251	24	<i>Scabiosa succisa</i>
S 231	2	<i>Mentha rotundifolia</i>	252	Sep. 3	<i>Colchicum autumnale</i> ¹
S 232	2	<i>Pastinaca sativa</i>	253	16	<i>Hedera helix</i>
S 233	3	<i>Melampyrum pratense</i>			

¹ Found near Hereford.

Alphabetical List, 1921

Name	Date	Name	Date
<i>Acer Pseudoplatanus</i>	Apr. 28	<i>Asperula cynanchica</i>	Ju. 27
<i>Achillea millefolium</i>	Ju. 30	<i>Asperula odorata</i>	May 7
<i>Adoxa Moschatellina</i>	Feb. 16	<i>Avena flavescens</i>	Ju. 25
<i>Ægopodium Podagraris</i>	May 20	<i>Avena pratensis</i>	May 30
<i>Æthusa Cynapium</i>	Ju. 26	<i>Ballota nigra</i>	Ju. 13
<i>Agrimonia Eupatorium</i>	23	<i>Bartsia Odontites</i>	30
<i>Ajuga reptans</i>	Apr. 23	C <i>Bellis perennis</i> (1920)	Nov. 17
<i>Ajuga reptans</i> (pink)	May 3	<i>Briza media</i>	Ju. 8
C <i>Alliaria officinalis</i>	Apr. 1	<i>Bromus arvensis</i>	May 25
<i>Allium ursinum</i>	13	<i>Bromus asper</i>	Ju. 28
<i>Alnus glutinosa</i>	Feb. 13	<i>Bryonia dioica</i> ♀	May 23
<i>Alopecurus pratensis</i>	Apr. 15	<i>Bryonia dioica</i> ♂	Ju. 5
<i>Anagallis arvensis</i>	Ju. 8	<i>Bunium flexuosum</i>	May 12
S <i>Angelica sylvestris</i>	Aug. 1	<i>Calamintha Clinopodium</i>	Ju. 30
<i>Anemone nemorosa</i>	Mar. 6	S <i>Calluna vulgaris</i>	July 31
S <i>Anthemis arvensis</i>	July 30	<i>Caltha palustris</i>	Feb. 14
<i>Anthemis Cotula</i>	Ju. 29	<i>Campanula rotundifolia</i>	July 30
<i>Anthoxanthum odoratum</i>	Apr. 26	<i>Campanula Trachelium</i>	Ju. 22
<i>Anthyllis vulneraria</i>	Ju. 5	<i>Capsella Bursa-pastoris</i>	Feb. 20
<i>Apium nodiflorum</i>	24	<i>Cardamine hirsuta</i>	Apr. 9
S <i>Arctium Lappa</i>	Aug. 1	<i>Cardamine pratensis</i>	Feb. 14
<i>Arrhenatherum avenaceum</i>	Ju. 17	<i>Carduus acanthoides</i>	Ju. 19
<i>Artemisia vulgaris</i>	July 30	<i>Carduus acaulis</i>	27
<i>Arum maculatum</i>	Apr. 9	<i>Carduus arvensis</i> ♀	20

Alphabetical List—*continued*

Name	Date	Name	Date
<i>Carduus crispus</i>	Apr. 16	<i>Hordeum pratense</i>	Ju. 24
<i>Carduus palustris</i>	May 21	C <i>Humulus lupulus</i>	July 11
<i>Carex pendula</i>	Apr. 25	<i>Hypericum hirsutum</i>	Ju. 17
<i>Carex sylvatica</i>	May 9	S <i>Hypericum perforatum</i>	Aug. 1
S <i>Caucalis infesta</i>	Aug. 5	<i>Hypochaeris radicata</i>	May 18
<i>Centaurea nigra</i>	Ju. 11	S <i>Inula Conyza</i>	Aug. 6
<i>Centaurea Scabiosa</i>	15	S <i>Jasione montana</i>	16
<i>Cephalanthera grandiflora</i>	6	<i>Lactuca muralis</i>	Ju. 18
<i>Chærophyllyllum sylvestre</i>	Jan. 27	<i>Lamium album</i>	Jan. 11
<i>Chærophyllyllum temulum</i>	Apr. 20	<i>Lamium Galeobdolon</i>	Apr. 9
<i>Chelidonium majus</i>	15	<i>Lamium maculatum</i>	Feb. 22
<i>Chlora perfoliata</i>	Ju. 18	<i>Lamium purpureum</i>	17
<i>Chrysanthemum Leucanthemum</i>	May 19	<i>Lapsana communis</i>	Ju. 18
<i>Circæa lutetiana</i>	Ju. 18	<i>Lathyrus pratensis</i>	8
S <i>Claytonia perfoliata</i>	Aug. 4	<i>Ligustrum vulgare</i>	8
S <i>Clematis vitalba</i>	6	<i>Linaria Cymbalaria</i>	Apr. 12
<i>Colchicum autumnale</i>	Sep. 3	S <i>Linaria vulgaris</i>	Aug. 1
<i>Convolvulus arvensis</i>	Ju. 13	<i>Linum catharticum</i>	May 30
<i>Convolvulus sepium</i>	July 3	<i>Listera ovata</i>	Ju. 6
<i>Cornus sanguinea</i>	Ju. 8	<i>Lolium perenne</i>	5
<i>Corylus Avellana</i>	Jan. 3	S <i>Lonicera Periclymenum</i>	Aug. 3
<i>Cratægus monogyna</i>	Apr. 30	<i>Lotus corniculatus</i>	May 14
<i>Crepis taraxacifolia</i>	May 29	<i>Lychnis diurna</i>	Apr. 14
<i>Cynosurus cristatus</i>	Ju. 1	<i>Lychnis Flos-cuculi</i>	May 10
<i>Dactylis glomerata</i>	2	<i>Lychnis Githago</i>	Ju. 30
C <i>Dipsacus sylvestris</i>	July 20	S <i>Lychnis vespertina</i>	July 28
<i>Daucus Carota</i>	Ju. 12	S <i>Lycopsis arvensis</i>	23
<i>Epilobium angustifolium</i>	12	S <i>Lycopus europæus</i>	Aug. 11
<i>Epilobium hirsutum</i>	26	<i>Lysimachia nummularia</i>	Ju. 13
<i>Epilobium montanum</i>	May 28	<i>Malva moschata</i>	25
<i>Epilobium parviflorum</i>	Ju. 22	<i>Malva sylvestris</i>	16
S <i>Erica cinerea</i>	July 31	<i>Matricaria Chamomilla</i>	9
S <i>Erodium cicutarium</i>	26	<i>Medicago lupulina</i>	Apr. 26
<i>Eupatorium cannabinum</i>	Ju. 22	S <i>Melampyrum pratense</i>	Aug. 3
<i>Euphrasia officinalis</i>	1	<i>Melica uniflora</i>	May 14
<i>Fragaria vesca</i>	Feb. 1	C <i>Melilotus officinalis</i>	July 7
<i>Fraxinus excelsior</i> ♂	Mar. 26	S <i>Mentha aquatica</i>	Aug. 6
<i>Fumaria officinalis</i>	May 27	S <i>Mentha rotundifolia</i>	2
<i>Galanthus nivalis</i>	Jan. 7	<i>Mercurialis perennis</i>	Jan. 24
<i>Galium Aparine</i>	May 17	<i>Milium effusum</i>	May 25
<i>Galium mollugo</i>	Ju. 15	<i>Nasturtium officinale</i>	10
<i>Galium verum</i>	23	C <i>Nepeta Glechoma</i>	Mar. 17
<i>Genista tinctoria</i>	16	<i>Oenanthe pimpinelloides</i>	May 25
<i>Geranium dissectum</i>	May 6	<i>Onobrychis sativa</i>	30
<i>Geranium phæum</i>	Apr. 29	<i>Ononis arvensis</i>	Ju. 25
<i>Geranium pratense</i>	Ju. 15	<i>Ononis spinosa</i>	29
<i>Geranium Robertianum</i>	Apr. 10	<i>Ophrys apifera</i>	5
<i>Geum urbanum</i>	May 1	<i>Ophrys muscifera</i>	5
<i>Hedera helix</i>	Sep. 16	<i>Orchis maculata</i>	May 21
<i>Helianthemum vulgare</i>	Ju. 3	<i>Orchis Morio</i>	4
C <i>Hieracium Sphondylium</i>	Mar. 28	<i>Orchis pyramidalis</i>	Ju. 6
<i>Hieracium murorum</i>	Ju. 3	<i>Origanum vulgare</i>	Aug. 6
<i>Hieracium Pilosella</i>	May 23	<i>Papaver dubium</i>	Ju. 5
<i>Holcus lanatus</i>	Ju. 3	<i>Parietaria officinalis</i>	1
		<i>Paris quadrifolia</i>	Apr. 10

Alphabetical List—*continued*

Name	Date	Name	Date
Pastinaca sativa	Ju. 19	Sedum Telephium	Aug. 10
S Pedicularis palustris	July 31	Senecio erucifolius	20
Pedicularis sylvatica	Apr. 11	Senecio Jacobæa	Ju. 12
C Petasites fragrans (1920)	Nov. 19	S Senecio sylvaticus	July 31
Phleum pratense	Ju. 26	Silauus pratense	Ju. 8
S Pimpinella saxifraga	Aug. 1	S Silene inflata	Aug. 2
Plantago lanceolata	Apr. 9	Sisymbrium officinale	May 8
Plantago media	May 23	Solanum dulcamara	28
Plantago major	Ju. 21	S Solanum nigrum	Aug. 1
C Poa annua	Mar. 22	S Solidago Virga-aurea	1
S Poa aquatica	Aug. 8	Sonchus arvensis	May 28
Poa pratensis	Ju. 14	S Sparganium racemosum	Aug. 11
S Polygonum convolvulus	Aug. 17	Spiræa ulmaria	Ju. 5
S Polygonum Persicaria	12	Stachys Betonica	July 1
Potentilla anserina	May 5	Stachys sylvatica	May 8
Potentilla reptans	Ju. 17	Stellaria Holostea	Apr. 9
Potentilla Tormentilla	May 4	C Stellaria media	Mar. 6
Poterium sanguisorba	Apr. 30	Tamus communis	May 15
C Primula veris (1920)	Nov. 17	C Taraxacum Dens-leonis (1920)	Nov. 14
C Prunus spinosa	Mar. 20	S Teucrium Scorodonia	July 20
Pyrola minor	Ju. 3	Thymus serpyllum	Ju. 3
C Pyrus Aucuparia	Apr. 4	Tragopogon pratense	May 27
Ranunculus acris	30	Trifolium incarnatum	13
Ranunculus aquatilis	15	Trifolium pratense	Apr. 28
Ranunculus arvensis	May 17	Trifolium repens	May 30
Ranunculus auricomus, <i>var.</i>	Apr. 10	Triticum caninum	Ju. 28
depauperatus		Tussilago Farfara	Feb. 16
Ranunculus bulbosus	May 1	Ulex europæus	8
Ranunculus Ficaria	Jan. 10	Ulmus montana	12
Ranunculus repens	25	Urtica dioica	May 22
Ranunculus sceleratus	May 13	Verbascum nigrum	Aug. 15
Reseda luteola	Ju. 3	Verbascum Thapsus	Ju. 17
Rhinanthus Crista-galli	May 15	Veronica agrestis	Feb. 17
Rosa canina	18	Veronica Beccabunga	May 12
Rubus cæsius	6	Veronica Chamædrys	Apr. 11
Rubus fruticosus	Ju. 18	C Veronica hederæfolia	Mar. 22
Rumex Acetosa	May 17	Veronica montana	Apr. 29
S Rumex acetosella	July 31	Veronica serpyllifolia	May 6
C Sambucus nigra	Apr. 4?	Viburnum lantana	Apr. 24
Sanicula europæa	May 2	Vicia cracca	Ju. 21
Scabiosa arvensis	30	Vicia sativa	May 6
Scabiosa columbaria	Ju. 19	Vicia sepium	Apr. 13
Scabiosa succisa	Aug. 24	Viola odorata	Mar. 6
Scrophularia aquatica	Ju. 8	Viola sylvatica	Feb. 23
Scrophularia nodosa	May 6	Viola tricolor	Apr. 17
Sedum acre	Ju. 27	Vinca minor	Feb. 8
Sedum album	27		

FURTHER OBSERVATIONS UPON THE MECHANISM OF ROOT PRESSURE

BY J. H. PRIESTLEY

IN a recent number of this journal, V. H. Blackman⁽²⁾ makes frequent critical reference to a recent attempt made by the present writer⁽¹¹⁾ to explain the mechanism of root pressure in terms consistent with the facts and with physical and chemical laws. Since the publication of the paper referred to the writer has continued the experimental study of exudation pressures and some results of these studies now await publication. In the meantime a brief re-statement of the main principles involved in the original hypothesis seems desirable, in view both of Blackman's criticisms and of other work to be mentioned later.

Reference to the earlier paper will show that the mechanism suggested requires the fulfilment of the following structural and physiological conditions if an adequate exudation pressure is to be maintained in the xylem vessels of the root.

1. A continuous chain of protoplasts with living semi-permeable membranes, permitting of the movement of water inwards to the protoplasts adjoining the xylem vessel by the ordinary process of osmosis.
2. A continuous supply of solutes to the xylem vessel to replace those carried upwards by the ascending column of sap.
3. An endodermis in which the network of Casparian strip performs two functions, viz. (a) It forms a rigid framework preventing too much extension of the tightly packed protoplasts within the endodermal cylinder. (b) It prevents leakage of the sap, and therefore of the solutes, from the xylem through the walls intervening between the protoplasts. As the protoplasts of the endodermis are themselves relatively impermeable, this structural peculiarity of the endodermis renders practically negligible the loss of solutes from the xylem into the cortex.

On all these features of the mechanism it is desired to add a few comments.

I. OSMOSIS AND WATER ABSORPTION

Blackman⁽²⁾, *loc. cit.* p. 111, is certainly justified in drawing attention to the neglect of Thoday's useful paper⁽¹³⁾. The phraseology adopted in that paper makes it admirably clear that the passage of

water from the root hair to the protoplast adjoining the xylem vessel depends upon a *gradient of absorbing* power and not of concentration of osmotic substances. Another serious omission in the earlier paper was the neglect to point out that Ewart(5) had already clearly shown that such a gradient of absorbing power might well arise as a result of the restriction placed upon the distension of the protoplasts adjoining the vascular strand.

The only positive contribution made by the present writer in this connection was to show that the mechanical structure of the endodermis was extraordinarily well adapted to facilitate the formation of such a gradient of absorbing power. This is merely an application of the earlier results of Schwendener(12) who investigated so thoroughly the mechanical properties of the endodermal cylinder. It is, however, essential to distinguish between different types of endodermis, as is indicated more fully later. The endodermis in the absorptive region of the root always possesses a well-marked Casparian strip but has no other strengthening devices. That the Casparian network itself is a relatively rigid structure is a fact that forces itself upon the botanical student at an early stage of his studies. As sections of roots are cut, the extra strain thrown upon the junction between rigid strip and elastic cellulose wall leads frequently to visible deformation of the wall in the neighbourhood of the strip. In many cases the whole central cylinder breaks away from the cortex, such breaks almost invariably occurring in the immediate vicinity of the Casparian strip.

2. THE SUPPLY OF SOLUTES

It seems desirable to state a little more fully the case for the mechanism providing the supply of solutes to the xylem. In the original statement use was made of a comparison with the excretion of sap from the capitate cells of superficial hydathodes, and certain experimental evidence was brought forward in support of this analogy. The data as to the condition for the excretion of sap by superficial hydathodes were supplied by the work of Lepeschkin(8). Blackman (*loc. cit.* p. 107) points out that the interpretation given by Lepeschkin of his experimental results is very much open to question and that the value of the work lies in the data supplied.

It is therefore emphasised that the only use made of Lepeschkin's work in the earlier paper (Priestley, *loc. cit.* p. 193) was to utilise his data. The only theoretical statement adopted from his work was that a different permeability of the protoplast membrane on different

sides of the cell seemed a necessary assumption to explain the facts obtained (Priestley, *loc. cit.* p. 195). This would certainly seem to be true for superficial hydathodes. In the case of the mass of parenchyma enclosed within the root endodermis however, the needs of the mechanism invoked will be adequately met if some of the protoplasts enclosed within the endodermal cylinder are relatively permeable to solutes, presumably organic solutes, to which other protoplasts within the cylinder are impermeable. Experimental evidence for the existence of these more readily permeable protoplasts has been briefly put forward, and certain other cogent reasons for assuming their existence will now be given.

The absorbing region of a root is always close behind the actively growing apex, and in this region, so long as conditions are suitable for the manifestation of root pressure they are also suitable for growth. At this distance behind the growing apex growth will be manifest as differentiation, and within the endodermal cylinder such differentiation involves the continual metamorphosis of normal protoplasts into empty xylem vessels and sieve tubes. These changes consequent upon differentiation must release a relatively large bulk of organic solutes which are unable to leak outwards to the cortex because of the functional endodermis and which must therefore find their way into the xylem. It does not seem at all improbable that this source of solute alone, always available whilst the root is growing, would be adequate to account for the normal exudation pressure developed within the root. When it is considered that such changes are proceeding in every active terminal branch of a large root system it is clear that they will supply a very large bulk of organic solutes to the ascending sap and so long as the root is growing the supply will be constantly maintained.

Reference may now be made to an earlier investigation upon root pressure which may throw an important light upon the condition under which protoplasts, bordering upon the already differentiated xylem, may gradually become increasingly permeable until their contents are allowed to diffuse into the xylem stream or are deposited upon their walls. Chamberlain(4), in an incomplete investigation, examined the effect upon the rate of flow of altering the pressure in the ascending sap column. Somewhat to his surprise, apparently, he found that if the root system was driving sap into a manometric system fixed on to the cut stem, when the pressure upon the sap was increased, the rate of flow after a temporary check, actually increased. Conversely if the pressure upon the head of sap

was decreased the rate of upward flow of the sap increased for the moment, but soon fell below its original value. Chamberlain regarded his results as inexplicable in terms of physics and chemistry and therefore as an argument in favour of "vital" activity in exudation pressures. Unfortunately, when critically examined, the data given by Chamberlain do not permit of a definite decision between two obviously possible explanations that involve no introduction of a mysterious "vital" force.

If the exudation mechanism, briefly outlined at the outset of the paper, be reviewed, it will be seen that the driving force for the upward flow of sap is the difference of osmotic pressure existing between the sap in the xylem vessel and the soil solution. The concentration of the soil solution may be regarded for the present discussion as constant; increased or diminished flow of the ascending sap will therefore be directly dependent upon increased or diminished concentration of solutes in the xylem vessel which are unable to pass the semi-permeable membrane of some of the surrounding protoplasts.

If therefore the sap in the xylem vessels be suddenly placed by experimental means under increased pressure, the temporary halt in the flow of sap as the pressures adjust themselves will be accompanied by an increased concentration of the solutes in the xylem, if their discharge continues as before whilst they are no longer carried away so rapidly. Obviously, therefore, after the root system has adjusted itself to the new pressure, the flow of sap will recommence and for a time at a greater rate. If, however, the more rapid flow is only due to the temporary accumulation of solutes, the final equilibrium rate, depending as it does upon the rate of leakage of solutes into the xylem vessel as balanced against their loss in the upward flow, should be practically the same as the rate of flow existing before the pressure was altered.

If, however, the rate of flow remains steadily higher when a steady rate is reached, then it would be clear that the concentration of solutes in the xylem sap was actually now maintained at a higher level although the rate of flow was greater. The natural cause to assign for this on a first analysis would be an increased permeability in some of the cells adjacent to the xylem vessel as a result of the increased pressure. This is a conclusion of very great significance and might well have great bearing upon the problem of the nature of the causal factors controlling xylem differentiation.

The converse argument would lead us to expect that a smaller flow under diminished pressure might be explained by a lessened

permeability of the protoplasts releasing solutes, when the pressure exerted on their surfaces through the xylem sap is diminished. This argument might have a direct bearing on the relatively rapid way in which the flow of sap ceases with many plants when the stems are cut off, although it is probably maintained in the normal plant for long periods and may become manifest at any time throughout the growing season. Unfortunately, an analysis of Chamberlain's data does not permit of any confident decision being reached as between these two alternative factors, both capable of accounting for an increased flow of sap under an increased pressure and a decreased flow with a diminished pressure.

3. THE STRUCTURE OF THE ENDODERMIS

The significance of the structural features of the endodermis will be fully considered in a later communication. As, however, the statements made in the original paper may evidently be seriously misapplied unless certain well-established facts are immediately pointed out, a later discussion is anticipated to this slight extent.

Kroemer(7) clearly defined the different structural stages through which the cells of the endodermis may pass. Originally normal meristematic cells, the endodermal cells then pass into a "primary" stage in which tangential walls are of cellulose and permeable to water and solutes, but radial and transverse walls are modified and rendered relatively impermeable by the presence of the Casparian strip. Later, all or some of the cells of the cylinder may develop a suberin lamella which renders them relatively very impermeable, as was pointed out by Schwendener(12). Such endodermal cells with suberin lamella Kroemer describes as in the "secondary" stage. Later, again, in the Angiosperm endodermis, a "tertiary" stage develops in which the cells contain, within the suberin lamella, later deposited thickening layers of cellulose.

In roots, in the absorbing region, the endodermal cells appear to be without exception in the "primary" stage and it is to this type of endodermis *only* that the discussion in the earlier paper applies. This warning seems necessary, as Bower(3) has recently applied these conceptions of endodermal functions in an interesting discussion of their influence upon the evolution of the stelar structure of Ferns. He describes the endodermal layer as placing the exchange of substances between vascular strand and ground tissue "under protoplasmic control." This is true for the primary stage endodermis only, but in the Leptosporangiate Ferns the endodermis of root, rhizome

and frond, save in the absorbing region of the root and in regions close behind growing apices, is already in the secondary stage (see Bäsecke(1)). In the Eusporangiate Ferns on the other hand, a primary stage endodermis is retained throughout life (Mager(9)); whilst throughout the Ferns a tertiary stage endodermis does not seem to occur.

In conclusion, brief reference may be made to one further point in the original paper to which Blackman takes exception.

It was pointed out that the water issuing from the hydathodes terminating leaf veins was sometimes practically pure water, although it seems most unlikely that any mechanism exists within the root likely to drive pure water into the xylem vessel. A reconsideration of the scheme originally suggested by Pfeffer(10) to explain the exudation of pure water, and which is re-discussed by Blackman (*loc. cit.* p. 112), only confirms the writer in the impression that a physiological mechanism acting in this manner is exceedingly difficult to visualise and so far has no experimental support. On the other hand, for the exudation of solutes into the xylem vessel and the resultant sap pressure, there is ample evidence, and ready comprehension of the physical mechanism involved. Unless, therefore, this hypothesis breaks down under employment it seems the natural one to adopt.

The exudation of pure water through the hydathodes terminating the veins seemed a possible example of failure of the hypothesis but on examination presents no difficulty. It was suggested that the solutes exuded into the vessel at its base might be withdrawn higher up as the result of adsorption, followed by "physiological absorption." Blackman considers the introduction of such a subsidiary hypothesis as seriously weakening the value of the theory. This surely depends upon the extent to which the subsidiary hypothesis is in accordance with the facts and the writer regrets again that he failed originally to draw attention to Ewart's(6) (*loc. cit.* pp. 349-351) earlier convincing experiments upon the adsorption of solutes during their passage along the xylem.

Further discussion of the question must await the publication of data, accumulated by the writer in conjunction with Miss Armstead, upon the removal of sugar during its passage in solution through the xylem. It may, however, be pointed out that the use of the phrase "physiological absorption" was not intended to convey the idea of any process more "unknown" than that which takes place whenever solutes, diffusing into cells, accumulate within them because they

are withdrawn from solution. If, for instance, sugar diffuses from a weak solution in the xylem vessel into the sap of a neighbouring protoplast and is then removed from solution because used in metabolism or stored as starch, then the diffusion gradient will continue to drive sugar into this relatively permeable protoplast and "physiological absorption" will be in progress.

In this connection it is difficult to see why Blackman lays so much stress upon the necessity of oxygen and suitable temperature relations for the manifestation of root pressure, as indicating that "vital" factors other than simple osmotic relations are involved. Surely this dependence of root pressure upon external conditions necessary for healthy existence may be completely explained by the fact that the semi-permeable membrane of the normal protoplast is only semi-permeable when healthy. And however adequate physically the definition of osmotic pressure given by Blackman (*loc. cit.* p. 108), it is necessary to remember that, for practical purposes, the manifestation of osmotic activity requires the presence of the so-called "semi-permeable" membrane.

BOTANICAL DEPARTMENT,
UNIVERSITY OF LEEDS,
December 1921.

REFERENCES

- (1) BÄSECKE, PAUL. Beiträge zur Kenntnis der physiologischen Scheiden der Achsen und Wedel der Filicinae. *Bot. Zeit.* **66**, p. 25. 1908.
- (2) BLACKMAN, V. H. Osmotic Pressure, Root Pressure, and Exudation. *New Phytologist*, **20**, pp. 106-115. 1921.
- (3) BOWER, F. O. Presidential Address to Royal Society of Edinburgh, 1920. *Proceedings*, **41**, p. 1. 1921.
- (4) CHAMBERLAIN, H. S. La Sève Ascendante. *Bull. du Laboratoire de Bot. Gén. de l'Univ. de Genève*, **2**, pp. 1-340. Neuchâtel, 1897.
- (5) EWART, A. J. On the Ascent of Water in Trees. I. *Phil. Trans. Roy. Soc.* **198 B**, p. 41. 1905.
- (6) ——— Ibid. II. *Phil. Trans. Roy. Soc.* **199 B**, p. 341. 1908.
- (7) KROEMER, KARL. Wurzelhaut, Hypodermis und Endodermis der Angiospermenwurzel. *Bibl. Bot.* **59**, p. 1. 1903.
- (8) LEPESCHKIN, W. W. Zur Kenntnis des Mechanismus der aktiven Wasserausscheidung der Pflanzen. *Beihefte zur Bot. Cent.* **19** (1), p. 409. 1906.
- (9) MAGER, HANS. Beiträge zur Anatomie der physiologischen Scheiden der Pteridophyten. *Bibl. Bot.* **14**, Heft 66. 1907.
- (10) PFEFFER, W. *Osmotische Untersuchungen*. (1877.)
- (11) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytologist*, **19**, pp. 189-200. 1920.
- (12) SCHWENDENER, S. Die Schutzscheiden und ihre Verstärkungen. *Abhand. der Königl. Akad. der Wissensch. Berlin*, **45**, pp. 3-75. 1882.
- (13) THODAY, D. On Turgescence and the Absorption of Water by the Cells of Plants. *New Phytologist*, **17**, p. 108. 1918.

RECORDS OF AUTUMNAL OR SECOND FLOWERINGS OF PLANTS

BY FRANCIS DARWIN AND A. SHRUBBS (Univ. Herbarium)

THE following observations on the autumnal or second flowering of plants were made in Cambridgeshire (C), Gloucestershire (G), and a few in Sussex (S). It can hardly be doubted that these second flowerings are connected with relatively high temperatures, the records of which are taken from the *Weekly Weather Report of the Meteorological Office*, and represent plus or minus deviations from the normal.

Name	1921	Temp. deviation	Name	1921	Temp. deviation
Lychnis Githago	Oct. 8 C	+ 11·6	Senecio Jacobæa	Oct. 29 C	+ 0·8
Bartsia Odontites	" C	"	Lolium perenne	" G	"
Lamium hybridum	Oct. 10 C	+ 7·3	Valerianella olitoria	" C	"
Veronica arvensis	Oct. 15 C	+ 7·3	Blackstonia perfoliata	" C	"
Veronica Tournefortii	" C	"	Senecio Jacobæa	Oct. 30 G	+ 3·7
Malva rotundifolia	" C	"	Heracleum Sphondylium	" G	"
Veronica agrestis	" C	"	Dactylis glomerata	Oct. 31 G	—
Potentilla anserina	Oct. 20 C	+ 5·3	Daucus Carota	" G	—
Corydalis lutea	Oct. 22 C	+ 5·3	Matricaria Chamomilla	Nov. 1 G	+ 3·7
Centaurea nigra	" C	"	Lamium purpureum	" G	"
Myosurus minimus	" C	"	Fragaria vesca	Nov. 2 G	+ 0·8
Papaver Rhæus	" C	"	Veronica agrestis	" G	"
Centaurea Scabiosa	" C	"	Cuscuta Epithymum	" C	"
Pulicaria dysenterica	" C	"	Cnicus arvensis	Nov. 3 G	—
Rosa arvensis	" C	"	Angelica sylvestris	Nov. 4 G	+ 3·7
Geum urbanum	Oct. 28 G	- 1·0	Chlora perfoliata	" G	"
Lychnis dioica	" G	"	Cnicus lanccolatus	" G	"
Ranunculus repens	" G	"	Rubus fruticosus	" G	"
Dipsacus sylvestris	" C	"	Blackstonia perfoliata	" G	"
Geranium Robertianum	" G	"	Solanum nigrum	Nov. 5 C	+ 0·8
Centaurea nigra	" G	"	Veronica hederæfolia	Nov. 8 C	- 9·2
Lamium album	" G	"	Veronica Beccabunga	Nov. 12 C	- 9·2
Trifolium pratense	" G	"	Myosotis arvensis	" C	"
Taraxacum officinale	" G	"	Euphorbia Esula	" C	"
Lychnis = (diurna)	" G	"	Linaria Cymbalaria	Nov. 14 C	- 1·3
Trifolium fragiferum	Oct. 29 C	+ 0·8	Alchemilla arvensis	" C	"
Lamium maculatum	" G	"	Ulex europæus	Nov. 15 S	- 2·8
Agrimonia Odorata	" C	"	Linaria vulgaris	Nov. 17 C	- 1·3
Lychnis alba (vespertina)	" G	"	Cardamine hirsuta	Nov. 22 C	- 1·3
Cornus sanguinea	" G	"	Erica cinerea	Nov. 26 S	+ 0·1
Veronica Anagallis	" C	"	Solidago Virga-aurea	Nov. 28 S	- 5·4
Arrhenatherum elatius	" G	"	Lamium album	Nov. 28 C	- 5·1
Capsella Bursa-pastoris	" G	"	Sonchus oleraceus	Nov. 31 G	
Potentilla reptans	" C	"	Mercurialis annua	Dec. 10 C	+ 3·0
Centaurea Scabiosa	" G	"	Lamium album	Dec. 12 C	+ 3·5
Bellis perennis	" G	"	Euphorbia Peplus	Dec. 20 C	+ 7·5
Senecio aquaticus	" C	"	Urtica urens	" C	"
Phleum pratense	" G	"			

THE NEW PHYTOLOGIST

VOL. XXI, No. 2

25 APRIL, 1922

PERMEABILITY

By WALTER STILES

CHAPTER VII

THE CELL WALL

IT is characteristic of the plant cell as contrasted with the animal cell that the living protoplasts are surrounded by what is generally regarded as a non-living envelope, the cell wall. Certain plant cells, such as some unicellular algæ, spermatozoids and egg-cells are without this envelope, while some animal cells possess a very definite membrane surrounding them, such as the unicellular *Vorticella* and the eggs of some marine organisms.

But quite apart from the cell wall which forms an undoubted membrane surrounding the protoplast, it is the general opinion of botanists and physiologists that a membrane surrounds the protoplast, separating the general body of it from the cell wall. To this supposed membrane a variety of names have been given, of which external plasmatic membrane, plasma-membrane and ectoplast are those most usually employed. A similar membrane, the internal plasmatic membrane, tonoplast or vacuole wall is also recognised (de Vries, 1885) and there are even supposed to be similar membranes around the nucleus and all cell inclusions (see *e.g.* Vonwiller, 1918). On the other hand, some workers, principally those interested in animal cells, deny the presence of such membranes altogether. As the nature of the cell membranes is obviously a very fundamental matter in regard to cell permeability, some little space will here be devoted to a consideration of the cell wall, while in the next chapter consideration will be given to the evidence for the existence of plasmatic membranes.

STRUCTURE AND COMPOSITION OF THE CELL WALL

Cell walls exhibit a great range in composition and structure according to the age of the cell and the tissue of which the cell forms a part. The cell wall which arises between the daughter cells after the division of a meristematic cell is always at first thin and apparently homogeneous in structure, and remains thin so long as the cells it separates are meristematic. But as conversion of the cells into part of a permanent tissue takes place, the cell wall increases in thickness, the material added in this process being apparently deposited in at least two different ways, known as growth by apposition and growth by intussusception. In the former fresh particles are added on the surface of the existing wall. Where this method takes the form of the deposition of entire discernible layers or lamellæ it is sometimes distinguished as growth by superposition. In growth by intussusception the new particles are deposited within the existing cell wall, so that they form an intimate mixture with the pre-existing particles. The method of thickening the cell wall may play an important part in determining its permeability.

Stratification of the cell wall is stated to be almost universal (Haberlandt, 1914). It is clear that this appearance must be due to differences in composition of adjacent layers, the differences being either physical, such as differences in water content (Nägeli, 1866) or in chemical composition.

In all cases it appears possible to distinguish between a layer forming the middle of the cell wall, *the middle lamella*, and layers on each side of this. It has been stated that the middle lamella in soft tissues is composed of a calcium compound of pectin, a name given to a group of substances which are nearly related to pentosans, that is, condensation products of pentose sugars. A substance or group of substances described as pectin or pectinogen (Schryver and Haynes, 1916) can be isolated from the walls of plants, and this substance can be converted by a clotting enzyme pectase into an acid, pectic acid, with splitting off of methyl alcohol and acetone (cf. Tutin, 1921). This acid forms soluble salts with the alkali metals, but an insoluble salt with calcium, and it is this salt which it has been supposed forms the middle lamella of parenchymatous cell walls. However, if plant tissue is treated so that all the pectin compounds, including the calcium pectate, are converted into pectic acid, and is then treated with a solution of ammonium oxalate or dilute sodium or ammonium hydroxide, the pectic acid dissolves, so that cells

separated by a pectic middle lamella fall apart. Such a maceration of tissue certainly takes place in many cases, but in many other cases it does not. Thus Professor Priestley informs me that in a bean stem after such treatment the epidermis, endodermis and cambium still remain as continuous layers, from which it is to be concluded that the radial walls of these cells do not possess a pectic middle layer. Professor Priestley also finds that the cells at meristematic apices of roots do not possess a pectic middle lamella, a very important finding.

The bulk of the cell walls in soft tissues is composed of cellulose, a group of substances which on hydrolysis yield glucose, and which are therefore to be regarded as glucosans. In addition to cellulose, or rather, celluloses, there may also be substances representing intermediate stages between cellulose and glucose, to which the substances termed "amyloid" appear to belong. Condensation products of other sugars such as mannose and galactose (mannosans and galactosans), included in the inappropriate term "hemicelluloses," may also be present, in addition to pectin. These substances are all complex carbohydrates, for information with regard to which reference may be made to standard chemical textbooks of the subject (*e.g.* Tollens, 1914; Haas and Hill, 1920). In these so-called cellulose walls other organic substances are also present. Thus Cranner (1914) has extracted fatty substances from the cell walls of the young parts of *Ricinus communis*, *Vicia Faba*, *Cucurbita Pepo* and a number of other species.

Apart from these organic constituents, the cell wall often contains a certain amount of mineral matter, silica, calcium oxide and calcium carbonate being the inorganic substances most frequently met with.

It is important for our purpose to know how these various substances found in the cell wall are there related to one another. It is clearly a feasible view to suppose the cellulose forming a solid framework, in the meshes of which the pectin and lipoid and other substances constitute the disperse phase of a hydrosol or hydrogel. This view, to which the writer inclines, would account for the rigidity and definitely solid character of the cell wall, and at the same time account for its powers of imbibition. But there are others who regard the cellulose itself as also forming part of the colloidal system. Thus Cranner, who thinks the fatty substances found by him in the cell wall are there probably in the form of salts of fatty acids, that is, as soaps, regards the cell wall as a hydrogel complex of which the more solid phase is composed of the colloid cellulose + pectin + colloidal soap. He was indeed able to prepare a compound of pectin and lauric acid and to manufacture membranes of this substance.

Cellulose walls are generally regarded as completely permeable both to water and the vast majority of dissolved substances, the chief evidence in support of this opinion being derived from the phenomenon of plasmolysis. This is the well-known contraction of the protoplast from the cell wall when the cell is immersed in a solution of higher osmotic concentration than that of the cell sap. Were the cell wall permeable to water but not to the dissolved substance in the external solution, the cell wall would constitute a semi-permeable membrane and so would be the contracting membrane to which the protoplast would continue to adhere.

However, if we accept the view which appears inevitable, having regard to the chemical composition of the cell wall, that the latter is, at least in part, a colloidal system, there appears every reason to suppose that the cell wall may play a part in determining the interchange of substances between the cell and its exterior. Thus Devaux (1901) showed by a spectroscopic method that the cell walls of a number of different species from different groups of the plant kingdom possess a strong absorptive capacity so that not only the ions of heavy metals such as iron, copper, silver and lead are absorbed, but also those of the alkalies and alkaline earths. A metal absorbed in the cell wall can be displaced by another. It will be recalled that relatively more substance is adsorbed from a weak solution than from a strong one, other conditions being equal, and Devaux has shown that absorption by the cell wall takes place from very dilute solutions. This author (1904) suggested that the absorption of substances by the thin walls of the root hairs facilitates the bringing of these substances into contact with the protoplasm of the root hair. Since Devaux treated his material with eau de Javelle, weak acid and pure water, Cranner points out that he must have removed the fatty acids and soaps from the cell wall, so that actually the absorptive capacity of the wall must be considerably greater than indicated by Devaux's results, for the lipid substances he removed are known to possess strong adsorptive properties. The assumption that the phenomena observed by Devaux are due to adsorption (cf. Czapek, 1913; Cranner, 1914) appears not altogether justified by the facts; they might be explained as due to chemical action.

In a series of experiments with parchment membranes and with membranes prepared artificially from cell walls, Cranner has shown that the amount of water absorbed by a cell wall membrane in unit time is influenced by the composition of the solution in which the membrane is immersed. The membrane takes up less water from a

0.02 *M* solution of calcium chloride than from solutions of sodium and potassium chlorides of the same molecular concentration. Membranes from which the lipid substance had been removed took up more water than similar membranes containing fatty constituents. Parchment membranes did not resemble cell wall membranes with regard to the influence of the composition of the surrounding solution on their uptake of water.

It is clear from these considerations that the cell wall cannot be dismissed as a negligible factor in cell permeability.

We have so far considered the cell wall of young active cells in which thickening has not taken place, or in which the thickening consists of "cellulose." But in many cases the cellulose walls become modified so that their permeability properties undergo much alteration. In some cases the change results in the wall becoming mucilaginous. It appears that in some species at any rate, the mucilage results from the conversion of polysaccharides into pentosans (condensation products of pentose sugars) which have great water-absorbing capacity (MacDougal, Richards and Spoehr, 1919). Other cell walls become lignified, suberised or cutinised (cuticularised). These changes are due to the deposition in the cell wall of substances, or rather mixtures of substances, known as lignin, suberin and cutin respectively. Among the substances which compose lignin a number have been isolated which are claimed to be characteristic of lignified walls; among these are xylan, lignic acids, and hadromal, an aromatic aldehyde (Czapek, 1899). Particularly important from the point of view of the student of permeability is the fact that in lignified walls the "cellulose" and lignin appear to be present in an intimate mixture (Robinson, 1920). This may partly account for the fact that water passes readily through lignified walls, but not through suberised walls.

Suberin is the name given to the group of substances to which the characteristic properties of cork are due. Various substances have been isolated from suberin, among these being the so-called suberogenic acids (Gilson, 1890). Some of these substances have been obtained pure and empirical formulæ obtained for them, *e.g.* phellonic acid, $C_{22}H_{43}O_3$, and phloionic acid, $C_{11}H_{21}O_4$ (after drying for weeks, $C_{20}H_{40}O_7$), while a third substance, suberinic acid, appears to possess the formula $C_{17}H_{30}O_3$. There appears to be a divergence of opinion on the fatty nature of suberin, but Gilson decides that the substances composing it cannot be regarded as true fats, an opinion with which Priestley (1921) agrees.

The significance of these suberogenic acids in regard to the im-

permeability to water of suberised walls is indicated by an experiment described by Priestley in which a Soxhlet thimble impregnated with a condensation product of potassium phellonate was found to be completely impermeable to water. It is significant in this regard also that according to van Wisselingh (1886-1892) the middle lamella of suberised walls consists of an unbroken layer of suberin, so that although cellulose is subsequently deposited on suberised walls, there is always present this continuous layer impermeable to water separating the cork cell from adjoining cells.

Cutin forms a continuous layer on the outside of the outer walls of the cells of leaves and herbaceous stems. Like suberin it is impermeable to water, and it is probably also like suberin in consisting of a mixture of several substances, although the evidence is not so definite. The cutinogenic acids obtained from cutin are, however, not the same as the suberogenic acids obtained from suberin.

With the chemistry of lignin, suberin and cutin we cannot deal further here. Those interested should consult the standard textbooks on the subject (especially Czapek, 1913) and the recent paper by Priestley already cited.

The coats of many seeds have a very low permeability to water. In some cases this is no doubt due to cutinisation or suberisation of cell walls, but in other cases to the presence of tannins, lipoid and pectic substances, as treatment of certain seed coats (*e.g.* pumpkin, almond, peanut) with solvents for these substances greatly increases the permeability (Denny, 1917*b*).

Before leaving the subject of the cell wall, it should be noted that the young cell wall is capable of stretching when acted upon by a force, while on removal of the force it tends to return to its original condition. It is thus elastic. With change in composition as the wall gets older this extensibility and elasticity may be lost, but cellulose walls generally retain extensibility and elasticity even when considerably thickened.

Mention has already been made of the pits in cell walls (*cf.* Chapter II). As the rate of diffusion through a membrane when diffusion is proceeding at a uniform rate varies inversely as the thickness of the membrane, it has been supposed that these pits help to expedite diffusion (Haberlandt, 1914) as the pit-closing membrane is considerably thinner than the thickened part of the cell wall. It is to be supposed in this connection that the relation between diameter of the pore and rate of diffusion through the pore found by H. Brown and Escombe (1900) also holds for the diffusion of substances in

solution, and that as the diameter of the pit is small the rate of diffusion through it is proportional to the diameter and not to the area of the pit, so that with decreasing area of the pit, the rate of diffusion decreases much more slowly. Similarly, the law for diffusion through a multi-perforate septum may also be presumed to hold.

The presence of chitin in the cell walls of Fungi has already been noted. For a summary of the literature dealing with the composition of the cell walls of these and other lower plants, the walls of which exhibit many differences from those of the higher plants, reference should be made to the first volume of Czapek's *Biochemie der Pflanzen* (1913), and for a description of the cell wall of some red algæ a paper by Sauvageau (1920) may be consulted.

SEMI-PERMEABLE CELL WALLS

Besides cell walls which allow the passage of water and most dissolved substances, and cutinised and suberised walls which are practically impermeable to water, there exist cell walls which are readily permeable to water, but impermeable, or almost so, to such simple solutes as sodium chloride (Gola, 1905). Such membranes were noted by A. J. Brown (1907) in the grain of barley. Barley grains readily absorb water from aqueous solutions of sulphuric acid, hydrochloric acid, cupric sulphate, ferrous sulphate, potassium chromate, silver nitrate and potassium ferrocyanide, while chemical analysis of the solutions shows that the dissolved substance is not absorbed. It thus appears that there is a semi-permeable membrane enclosing the seed of barley. That this membrane is not a living protoplasmic one becomes clear from the fact that grains can exhibit the semi-permeable action in strong solutions of such poisons as cupric sulphate and silver nitrate, which would inevitably destroy the protoplasm and its semi-permeable properties if they came into contact with it, while conclusive proof is obtained from the fact that grains killed by boiling still exhibit the property.

It is a curious fact that iodine, in solution in potassium iodide, is able to penetrate the membrane without apparently injuring it, for the subsequent passage of sodium thiosulphate is still prevented. Nitric acid, on the other hand, appears to destroy the membrane, for this acid is excluded for a time, but subsequently enters the grain.

Further evidence of the presence of a semi-permeable membrane in the grain of barley is given by the fact that the blue pigment contained in the aleurone cells of the grain of *Hordeum vulgare*, var. *cærulescens*, undergoes no change in colour when steeped in a solu-

tion of sulphuric acid, while in the presence of acid the pigment changes in colour to red.

In a subsequent paper A. J. Brown (1909) showed that less water is absorbed by barley grains from salt solutions than from pure water, a result to be expected if the covering of the grain contains a semi-permeable layer (compare Chapters VI and VIII).

Such semi-permeable membranes formed by cell walls have been shown by A. J. Brown (1907) to be present also in grains of *Avena*, *Secale* and *Triticum*, the semi-permeable membrane of the last named having also been investigated by Schroeder (1911). Gassner (1915) also records the presence of semi-permeable cell layers in seeds of Gramineae, while they have been observed by Tjebbes (1912) in sugar-beet seed, by Shull (1913) in the seeds already mentioned and in apple, pear, *Vicia Faba* and other Leguminosae, *Helianthus annuus*, *Xanthium glabratum* and *Alisma plantago-aquatica*, and by Rippel (1918) particularly in *Æsculus hippocastanum* and to a less extent in a number of other species. Rippel soaked seeds in a solution of N/10 sodium chloride, measured the uptake of water by weighing the seeds, and the intake of sodium chloride by titrating the solution against silver nitrate. He showed that generally speaking the greater the intake of water, the greater the intake of salt. His results are summarised in Table XVII.

TABLE XVII

Relation of Semi-permeability of Seeds of Various Species
to Water Intake (from Rippel)

Species	Water uptake %	Ratio of concentration of external solution to the theoretical concentration at the end of the experi- ment if the seeds are completely semi- permeable
<i>Trifolium pratense</i>	141.2	56.6
<i>Sinapis alba</i>	118.0	64.3
<i>Phaseolus multiflorus</i>	106.4	90.0
<i>Pisum sativum</i> I	87.4	82.5
" " II	83.0	85.9
<i>Vicia Faba</i>	65.2	91.3
<i>Lychnis Githago</i>	60.0	98.4
Rye	48.5	94.3
<i>Cannabis sativa</i>	48.2	95.8
<i>Æsculus hippocastanum</i>	21.8	100.0

Rippel suggests that these results may be explained on the view that the swelling of the seed by absorption of water ruptures the semi-permeable membrane.

With regard to the position of the semi-permeable layer very diverse opinions have been expressed. From the experiments with *Hordeum vulgare*, var. *cærulescens*, and by following the penetration of sodium chloride by silver nitrate, A. J. Brown thought it possible that the walls of cells derived from the nucellus form the semi-permeable membrane in barley grains. Schroeder in the case of wheat suggested the possibility of a localised entrance of water and dissolved substances into the grain, or possibly decreasing permeability of the coverings from the basal to the apical end of the grain. Reichard (1909) correlated the presence of a tannin layer in the barley grain with semi-permeability. Shull (1913), from his investigations on a number of species, particularly *Xanthium glabratum*, concluded that cellulose walls may form semi-permeable membranes, and stated that "the possible semipermeable character of cellulose membranes cannot be overlooked in future investigations dealing with the entrance of salts into plant tissues."

Shull disposed of Reichard's view that tannin determines the semi-permeable character of a layer in the seed coats of Gramineae by extracting the tannin from grain with sodium hydroxide without affecting the semi-permeable properties, while Collins (1918) was unable to confirm the presence of a tannin layer in barley grains.

The question appears to have been solved for the case of the barley grain by Collins who, in a careful morphological and physiological investigation, showed that the barley grain is completely surrounded by a strongly cutinised membrane except in the region of the micropyle and the chalaza. Only a very small proportion of the water absorbed by the grain can pass the cutinised layers, while dissolved substances are also kept back. The seat of rapid entry of water is at the micropylar region of the grain, and here must be the seat of semi-permeability. Entry of water through the chalaza appears much slower. The cells in the micropylar region (the so-called embryonic appendage) through which water must pass, appear possibly to be modified in the direction of pectic, mucilaginous, or gum compounds. The conclusion is not definite, and so cannot be used as evidence either for or against Shull's opinion of the possible wide distribution of semi-permeable cellulose membranes in plants.

From this review of the properties of cell walls with regard to permeability it is clear that we may find walls with very different degrees of permeability both to water and to various dissolved substances.

(To be continued)

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

I. INTRODUCTION

By J. H. PRIESTLEY

IN the general title to this series of papers the phrase "Physiological Anatomy" has been avoided because of the significance it bears in Haberlandt's great text-book, where a structure is regarded as explained when it has been shown to be efficient for the performance of its physiological duties. The great series of data thus marshalled by Haberlandt appear to be rather anatomical contributions to the understanding of plant physiology than contributions made by physiology to plant anatomy.

It is true that, theoretically, Natural Selection may explain the existence of any useful working mechanism, but the mere demonstration of usefulness tends to draw a veil over the way in which the mechanism comes into being, and any real explanation of the structure must wait until its development has been traced and interpreted in terms of physico-chemical causation. Such developmental studies will require an extensive re-investigation of the problems of anatomy, undertaken largely from a physiological point of view, and it is in this sense that the following series of papers may be regarded as physiological studies in plant anatomy.

The standpoint is not a new one; it has been expressed on several occasions by Lang(1), but so far as the writer is aware this is the first time it has been consistently applied to the detailed study of the development of a complex plant organism. From this standpoint, that of causal anatomy, a structure is not explained because it is adapted to its surroundings when mature, but as the inevitable outcome of the reaction between earlier developmental stages and its environment. Given these inter-related factors its development was inevitable, whether the mature structure is useful or not. That it is usually a useful and efficient structure remains, as before, an argument for the effectiveness of Natural Selection. It is obvious that only a beginning can be made upon so vast a field and it is not claimed that the few results obtained from the study of a limited number of plants are necessarily of general application. The experience gained so far in this field of investigation makes it possible to define some of the general questions more clearly than would have been possible

a year ago. These will now be stated in terms of the problem of the causal anatomy of the Angiosperm, although in later papers data are utilised from any group of vascular plants.

In the Angiosperm seedling certain internal factors are already present which mould subsequent development; these have been determined, either genetically, or during the maturation of the embryo within the developing seed. Thus, whether the seed is endospermic or non-endospermic, there is always an equatorial region of food supply, directed through an intermediary axis towards two polar, opposed meristems. These apical meristems already show different tropistic reactions and developmental characteristics which serve to distinguish stem and root. A later paper (IV) shows that in certain growing points more closely studied, the physiological differences between the apical meristems have a suggestive relation to the morphological divergence between stem and root. These are connected with the effect of the structure of the meristem upon the conditions under which food is supplied to support further growth.

As the problem of this supply of food to merismatic tissues arises over and over again in the course of the work, attention is drawn to certain relevant considerations of a general nature. Reasons are given later for regarding a merismatic cell as remaining non-vacuolated only so long as it is actively engaged in the synthesis of protoplasm, although connected with the rest of the plant by a tract of vacuolated cells. Changes in the permeability of this cell allowing entry of water, mean vacuolation, a thickening of the wall and, most important of all, the loss of merismatic activity. The actively growing merismatic cell must therefore be able to obtain the necessary nutriment from the solutes in the sap bathing its protoplast, but at the same time it must prevent the entry of water in which these solutes are dissolved; a problem which is considered in detail later (IV).

For the moment the problem is the source of the necessary organic solutes. The only channel for their passage is along the walls of the vacuolated cells bordering the meristem, there being usually no intercellular spaces immediately abutting upon a meristem. Water will always be moving along the walls of these vacuolated cells in the adjustment of the constantly changing equilibrium between protoplast and turgid wall, but if organic solutes are withdrawn from this water by the adjacent meristem there is little likelihood of a renewal of the supply by outward diffusion from these recently vacuolated protoplasts. The only possible source, therefore, of a constant and

unremitting supply of such solutes to the meristem is to be found in the sap from the neighbouring vascular strand. These solutes cannot diffuse from vascular strand to meristem, however, across a functional endodermis (III).

Consideration of later experimental results (II and V) suggests that the sap in the vascular strand is only likely to contain the necessary organic solutes when it is under pressure. It was the study of the development of such exudation pressures in the vascular strand (II) which first led to an investigation of their effect upon the growth of meristems. The most efficient organ in their production is the root, and reasons have been given (Priestley(3)) for considering that the endodermis plays an important *rôle* in enabling the root to develop and maintain these exudation pressures. A fuller study has therefore been made of the endodermis, and although the developmental side of this problem is still far from clear, a consideration of the results of two later papers (III and VI) certainly suggests that the sequence of events in the development of the endodermis follows naturally from the composition of the apical meristem and the interaction of the tissues just behind the meristem with the external environment.

A functional endodermis, by restraining within a limited area the supply of sap necessary for continuous merismatic growth, exerts a profound effect upon the future development of the plant. Thus the presence or absence of an endodermis seems to be responsible in part for the endogenous or exogenous origin of the lateral organs characteristic respectively of root and stem.

Two anatomical questions have enabled this view as to the significance of a functional endodermis to be submitted to a fairly severe test, from which it appears to emerge with credit.

(1) The occurrence of the meristem of periderm at various depths within the tissue of root and stem, as also its occurrence at leaf-fall or in response to injury, is analysed as fully as possible (V), and adequate causal mechanisms can be found accounting in all cases for the development of sap pressures prior to the origin of the phellogen. A striking correlation can be traced between the position of the phellogen and the presence or absence of a functional endodermis.

(2) It is shown (VI) that the shoot apex of some vascular plants, when etiolated, that is, when grown under conditions normal for a root, develops structural features more characteristic of a root. The lateral organs, the leaves, are much suppressed, and within the stem a functional endodermis, absent in the normal plant, encloses the

vascular cylinder from the base to within a few millimetres of the growing point. It is shown that the presence of this endodermis, restricting the flow of sap which normally irrigates the cortex, explains many of the morphological and structural peculiarities exhibited by these particular plants under etiolation conditions.

In conclusion the writer would like to make certain acknowledgements; firstly to the many other workers in the Botanical Department at Leeds, both staff and students, whose names are not associated with the papers published below, but whose active cooperation has been freely given and fully utilised throughout.

Secondly, whilst slowly developing the theory of the significance of sap movement to growth, the writer is very conscious that his views have been partly inspired by the suggestive experiments and writings of Jacques Loeb. His papers, cited below (2), have a general relation to the present line of thought, although these investigations have developed quite independently, and specific reference to Loeb's work has seldom proved necessary when setting down the results contained in the subsequent papers in this series.

Further Papers in the series, to appear in this Journal.

II. The Physiological Relation of the Surrounding Tissue to the Xylem and its Contents. J. H. PRIESTLEY and DOROTHY ARMSTEAD.

III. The Structure of the Endodermis in Relation to its Function. J. H. PRIESTLEY and EDITH E. NORTH.

IV. The Water Relations of the Plant Growing-Point. J. H. PRIESTLEY and R. M. TUPPER-CAREY.

V. Causal Factors in Cork Formation. J. H. PRIESTLEY and LETTICE M. WOFFENDEN.

VI. Etiolation. J. H. PRIESTLEY and J. EWING.

REFERENCES

- (1) LANG, W. H. A Theory of Alternation of Generations in Archegoniate Plants based upon the Ontogeny. *New Phytol.* 8, pp. 1-12. 1909.
 ——— Presidential Address, Section K. *British Association Report*, Manchester. 1915.
- (2) LOEB, JACQUES. Rules and Mechanism of Inhibition and Correlation in Regeneration of *Bryophyllum Calycinum*. *Bot. Gaz.* 60, p. 249. 1915.
 ——— Law Controlling the Quantity of Regeneration in the Stem of *Bryophyllum Calycinum*. *Journ. Gen. Physiol.* 1, p. 81. 1919.
 ——— Quantitative Laws in Regeneration. I. *Journ. Gen. Physiol.* 2, p. 297. 1920.
 ——— Quantitative Laws in Regeneration. II. *Journ. Gen. Physiol.* 2, p. 651. 1920.
 ——— Physiological Basis of Polarity. I. *Journ. Gen. Physiol.* 1, p. 337. 1919.
 ——— Physiological Basis of Polarity. II. *Journ. Gen. Physiol.* 1, p. 687. 1919.
- (3) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytol.* 19, pp. 189-200. 1920.

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

II. THE PHYSIOLOGICAL RELATION OF THE SURROUNDING TISSUE TO THE XYLEM AND ITS CONTENTS

By J. H. PRIESTLEY AND DOROTHY ARMSTEAD

CONTENTS

	PAGE
Introduction	62
I. Experiments with Stains and Living Tissues . . .	63
II. Exudation Pressures in Stem and Leaves . . .	64
III. Solutes in the Xylem Sap	68
IV. Exchange of Sugar between the Xylem and the Surrounding Protoplasts	73
Discussion	77
Summary	79

INTRODUCTION

AN account has recently been given (Priestley (20)) of a theory of the mechanism by which exudation pressure may be developed by a root system. In the present paper some fresh experimental evidence is presented which is relevant to this theory and the experimental investigation is extended to the study of such exudation pressures in stem and leaf. It is therefore necessary at the outset to re-state very briefly the essential features postulated for the root-pressure mechanism, viz.

(1) A xylem strand, which contains rigid permeable tubes, providing an open channel for the flow of sap.

(2) A supply of solutes to the xylem which maintains the sap at higher osmotic concentration than the dilute solution bathing the protoplasts at the surface of the root.

(3) The maintenance of this supply of solutes requires

(a) The presence of a functional endodermis, that is an endodermis whose radial and transverse walls are so relatively impermeable that the outward leakage of the solutes is prevented.

(b) The constant diffusion of solutes into the xylem sap from relatively permeable protoplasts within the endodermal cylinder.

The next paper in this series will examine the structure of the endodermis in relation to this mechanism, for the present we are

concerned only with the question of the provision of the solutes in the xylem sap. Certain experimental evidence based on vital staining has been given (Priestley, *loc. cit.*) for the existence of relatively permeable protoplasts that might prove a possible source of these solutes. This evidence is first briefly reconsidered. Such relatively permeable protoplasts may be found throughout the vascular strand, and the next section of the paper is devoted to experiments demonstrating the development of exudation pressures by stem or leaf, when placed under suitable conditions.

Subsequently data are given as to the quantity and nature of the solutes present in the xylem, and the possibility examined of the re-absorption of these solutes as they move along the vascular strand. There is, finally, a short discussion of the significance of these exudation processes as part of the mechanism concerned with the rise of water in the plant.

I. EXPERIMENTS WITH STAINS AND LIVING TISSUES

The essential idea of these experiments is that the penetration of the complex molecule of a dye into a protoplast may give some indication of the permeability of the membrane of that protoplast, especially if subsequent experiment shows that the stained protoplast is still capable of plasmolysis. In this case the protoplast membrane evidently retains its semi-permeability and Osterhout has shown(17) (*loc. cit.* p. 144) that this behaviour is a good index of its continued vitality.

In the earlier paper (Priestley, *loc. cit.* p. 193) it was shown that certain cells internal to the endodermis were exceptionally permeable to various dyes introduced into the xylem, and the analogous behaviour of the apical cells of multicellular excretory hydathodes was emphasized. Further work has confirmed these earlier observations and the experiment has withstood the test of repetition by a large class of students.

A wider experience of experiments of this type has brought to light one very interesting fact. When acid and basic dyes are used a comparison of the results will often reveal striking differences. Thus with *Vicia Faba* L. if 0.1 per cent. solutions of acid green or light green F.S., both acid dyes, are drawn up the xylem vessel of the root, the protoplasts of certain cells of the pericycle, especially the cells just opposite the protoxylem group, will be stained. If malachite green, a basic dye, be used instead, these protoplasts will

remain unstained whilst the protoplasts of cells immediately adjacent to the xylem will be stained.

Experiments upon the same plan show the presence of readily permeable protoplasts around the vascular strand in the stem also, and in so far as this method can be trusted it supplies definite evidence that various cells bordering upon the xylem strand throughout its course show such permeability to dyes that they may be expected to permit the passage of various solutes to the sap within the xylem.

II. EXUDATION PRESSURES IN STEM AND LEAVES

Wherever the xylem contains solutes, if the protoplast of the surrounding tissues are in contact at the exterior with a water supply less concentrated than the sap within the xylem, an exudation pressure should presumably be generated. In the root the consequence of such exudation pressures must be an upward stream of sap because the closure of the xylem channel at the root apex prevents all movement in that direction.

Under suitable conditions, therefore, it should be possible to demonstrate the existence of exudation pressures in stem or leaf. Thus if a piece of stem be sealed at one end and immersed in water, sap under positive pressure might be expected to emerge from the open end of the xylem strand. Such an exudation pressure might well be only temporary, as in Angiosperms there is usually no functional endodermis in stem or leaf, so that leakage of solute from the vascular strand will be continuous, and the cause of the exudation pressure thus gradually disappear.

In the first experiment a piece of stem of *Helianthus annuus* L. was stripped of its cuticle, one end dipped into melted wax and the whole piece subsequently immersed in water. The open end was attached by rubber tubing to a piece of capillary glass tubing, the end being freshly cut and the tubing attached under water, so that the experiment started with a water meniscus visible in the capillary tube. After twenty-four hours the meniscus had sunk out of sight beneath the rubber tubing owing to absorption of this small quantity of water by the cells around the xylem, and the apparatus was nearly dismantled; but, fortunately, it was left for forty-eight hours longer, when the meniscus was again seen, and rose rapidly to the top of the capillary tube to fall again a few days later, presumably owing to the outward diffusion of solutes from the xylem. This led to a whole series of further experiments with various stems, both herbaceous

and woody, and also with leafy twigs, etc. Particularly vigorous exudation pressure was developed by leafy twigs, *Syringa* (*Philadelphus*), species of *Ribes*, and *Syringa vulgaris* L., amongst many others, when inverted and immersed in water. With both stems and leafy twigs, instead of stripping off the cuticle or bark to enable the water to enter it was found sufficient to expose the stem and leaves to chloroform vapour for a minute or two. As a result the surface tension conditions at the stomata or lenticels were so modified that the water readily displaced the air and entered the stems at these points.

Later reference to the literature showed that all the experiments of Pitra (18) had been repeated and his observations of 1878 confirmed in practically every respect. This does not seem time wasted as Pitra's experiments are but little familiar to the present generation of botanists.

The following points in our experiments seem worth more detailed description as they extend Pitra's observations in some slight degree. Boehm (4) criticised Pitra's observations and explained the exudation of liquid as being due to gas developed in the xylem by fermentation under the anærobic unhealthy conditions in which the stems were placed. It is true that owing to the duration of many of Pitra's experiments fermentation effects were very possible, and our attention had been drawn to them by obvious fermentation effects in our own experiments when of relatively long duration. However it is quite possible to get the effects seen by Pitra under sterile conditions. As already stated, our stems were usually placed in chloroform vapour before mounting in water. This treatment could be rendered quite drastic, exposure for several hours to chloroform being without effect upon subsequent results. The vapour works inwards very slowly and quite thin stems require exposure for 48 hours to kill them. Stems then after rigorous treatment in chloroform were mounted under boiled distilled water in closed vessels, the stem emerging through a sterilised cork, and the tube above it containing a little sterilised water surmounted by a layer of toluol. Under these conditions the rise of liquid is still obtained and Boehm's objections seem to be disposed of by experimental evidence. The fact that in many cases vigorous exudation of sap is obtained in less than 24 hours also supplies strong evidence against the validity of Boehm's criticism.

Copeland (7) (*loc. cit.* p. 174) had already shown that Boehm's objections were probably not well founded on theoretical grounds,

and that in all probability Pitra's observations must find their explanation in osmotic phenomena. Stems killed by prolonged exposure to chloroform or by exposure to steam show no exudation pressure, which indicates that the phenomenon is dependent upon the living semi-permeable membranes of the protoplasts around the xylem. Copeland's views receive further justification in the results of a simple experiment several times repeated of which two examples are cited.

Lilac stems with leaves attached were set up with capillary tubes attached to their cut ends. Behind the capillary tube a glass scale was clamped. The level of the meniscus was read at frequent intervals by means of a telescope. After about an hour, the water from the glass vessel in which the stems were placed was siphoned off, and replaced by 5 per cent. salt solution, the level again read at intervals, and then the salt again replaced by water. In this way any change in the amount of liquid excreted into the xylem from the surrounding parenchyma and passed up into the tube, could be ascertained.

The movement of the meniscus is illustrated by the figures shown below:

Twig in water	
Time hrs. mins.	Height of meniscus
2.30 p.m.	2.25 cms.
2.34 "	2.30 "
2.38 "	2.38 "
2.42 "	2.42 "
2.46 "	2.47 "
2.54 "	2.60 "
3.0 "	2.70 "
3.26 "	3.00 "
3.32 "	3.20 "
3.32 water replaced by salt solution.	
3.45 p.m.	4.25 cms.
3.49 "	4.45 "
3.56 "	4.40 "
4.0 "	4.15 "
4.4 "	3.90 "
4.10 "	3.50 "
4.14 "	3.20 "
4.38 "	1.80 "
4.46 "	1.30 "
4.50 salt solution replaced by water.	
4.55 p.m.	1.0 cm.
4.57 "	1.20 cms.
5.4 "	1.70 "
5.18 "	2.70 "
5.30 "	3.40 "
5.52 "	3.90 "

Similar experiments were carried out with the petiole of *Spiræa*, sealed at the base with paraffin wax, and a capillary tube attached at the top cut end.

Petiole in water

Time hrs. mins.	Height of meniscus
10.30 a.m.	4.65 cms.
10.45 „	4.65 „
11.15 „	5.65 „
11.25 water replaced by salt solution.	
11.27 a.m.	4.40 cms.
11.31 „	4.31 „
11.35 „	4.20 „
11.41 „	4.05 „
11.46 „	3.95 „
12.51 p.m.	3.20 „
12.52 salt solution replaced by water.	
12.56 p.m.	3.00 cms.
1.0 „	3.05 „
1.51 „	4.05 „
2.4 „	4.25 „
2.30 „	4.45 „
3.12 „	4.65 „
3.40 „	3.95 „
3.51 „	3.95 „
4.6 „	3.90 „

These typical results show that the rise of liquid in the tube in this class of experiment is controlled by the concentration of solute in the medium external to the plant and is therefore due to osmotic phenomena.

Pitra's earlier observations are therefore confirmed, and without accepting all his conclusions, his general contention is endorsed that stems, like roots, possess the power of exhibiting exudation pressure. A long series of experiments directed to the same end was carried out by Kraus(16), whose usual method was to place cut twigs in damp sand and observe for what length of time they continued to bleed from various tissues at the upper end, where these tissues were exposed at cut surfaces. His experiments require one further comment. He remarks how frequently the bleeding is more active from other tissues (such as the pith and cortex) than from the xylem. A little reflection will show that under his experimental conditions this was to be expected. The organic solutes always diffuse from certain parenchymatous cells into the surrounding walls or intercellular space. In the uncut stem these ultimately find their way into the xylem as also does any water withdrawn from the proto-

plasts by the osmotic action of these solutes. In Kraus' experiments, these solutes were free to gather at the cut surface of the parenchymatous tissues, whilst such sap as collected in the xylem was sinking through the vessels into the moist sand below.

In concluding this section reference may be made to an incidental observation.

One of the earliest leafy twigs to be used for experiments was a branch of Fuchsia and negative results were obtained both on this occasion and whenever this plant was used. It seemed possible that the regularity with which the liquid sank in the tube above the cut end of the leafy Fuchsia branch might be due to the constant leakage proceeding through the multicellular hydathodes to be found at the termination of the xylem in the marginal teeth of the leaves. These hydathodes are known to exude liquid when the sap in the xylem is under positive pressure and it may well be that, simply under the head of water above the hydathode, the sap is flowing through the xylem out from the hydathodes into the water in which the leaves are immersed. Fuchsia twigs were therefore taken and the margin of the leaves dipped in melted paraffin wax. When now immersed in water, the rise of sap in the capillary tube above the cut end was noted after a few hours.

The experiments described in this section, then, together with the literature cited, seem to supply a considerable body of evidence for the existence under suitable conditions of an exudation into the xylem cavity from the surrounding parenchyma in stem and leaf as well as in root. This exudation would probably depend upon the two factors defined in a previous paper (Priestley (20)), (1) Osmotic withdrawal of water from parenchyma cells incapable of further extension and ultimately supplied from a watery medium less concentrated in solutes than the sap within the xylem vessel; and (2) A supply of solutes to the xylem sap adequate to maintain this relatively high osmotic concentration. This leads to a consideration of the solutes present in the sap of the xylem under different conditions, a question which is discussed in the succeeding section.

III. SOLUTES IN THE XYLEM SAP.

Gelston Atkins (1) (*loc. cit.* p. 187) has already pointed out how widespread is the erroneous impression that the water in the xylem vessel is an exceedingly dilute solution of inorganic salts, and the work emanating from the Botanical Department of Trinity College,

Dublin, and summarised in the pages of Atkins' monograph, has gone far to dispel this impression.

When exudation pressure is being manifested the necessary osmotic concentration must be maintained by solutes unable to escape across the endodermal barrier. It is unlikely, therefore, that these solutes will be inorganic salts which must in the first place diffuse into the root through the protoplasts of the endodermis. The nature of the organic solutes, whose leakage into the xylem might be anticipated, has been discussed elsewhere (Priestley, *loc. cit.* p. 197) and the conclusion reached that sugars and organic acids might be expected. The summary by Gelston Atkins already referred to gives an effective demonstration that sugars are usually present in the xylem sap, and in the course of our own experiments some quantitative estimations were made that support these statements.

Experiments with the Vine (Vitis vinifera L.)

Three large healthy vines in a temperately heated glasshouse, with their leaves well out, were selected. These were cut down at a level of about 9 inches from the ground. After chopping the stem through, the cut surface was levelled with a sharp knife, and any bits of splintered wood removed. To the stump was securely bound and wired a piece of an inner tube of a motor-cycle tyre and with this was connected a piece of glass tubing of two-inch bore.

The vines were cut down on March 24th; one pumped up an average of (over) 500 c.c. sap per day for 36 days, after which the amount gradually decreased. The other two gave an average of 250 c.c. per day, but had practically stopped in 28 days.

The liquid which collected in the wide glass tubes was siphoned off at intervals. The first fraction collected was found to go cloudy on standing 24 hours, owing to the presence of bacteria, and subsequent fractions were collected under toluol. This was effected by pouring a little toluol down the glass tube, the top of which was then plugged with cotton-wool to stop excessive evaporation. As the liquid accumulated in the tube it was in this way always under a layer of the antiseptic. When the fractions were siphoned off a little liquid plus the toluol was always left in the bottom of the tube so that the layer of toluol never came in direct contact with the cut surface of the stump. The liquid was kept in Winchester under a layer of toluol, and by this means was kept quite sterile.

With this liquid quantitative investigations were carried out, to find the amount of organic substance present.

The estimations of the sugar content were made volumetrically with standardised Fehling's solution, using potassium ferrocyanide in acetic acid as an indicator of the end point of the reaction (Clowes and Coleman (5), *loc. cit.* p. 354).

A known volume of the liquid was then inverted with hydrochloric acid for about 15 minutes in a water bath, cooled, neutralised with caustic soda, made up to a known volume and again titrated, to see if any further reduction took place after inversion.

An estimate of the total amount of solute present was made by evaporating a measured amount of the liquid to dryness in a weighed evaporating basin. This residue gave the weight of the inorganic and organic solutes present; it was then heated more strongly till the weight was constant, giving the weight of inorganic substances present.

The following results were obtained:

(1) *Estimation of sugars by the reduction of Fehling's solution:*

Before inversion	After inversion	Hexose	Cane sugar
0.136 per cent.	0.147 per cent.	0.136 per cent.	0.01 per cent.

Total residue on evaporating to dryness: 0.272 per cent.

Organic residue 0.206 per cent.

Inorganic „ 0.066 „

From these results it will be seen that there is evidently some other organic substance present in addition to sugars.

A portion of the sterile juice was therefore evaporated to dryness and the organic residue tested for *organic nitrogen* by heating with metallic sodium. The hot tube was then plunged into cold distilled water, which was then filtered and tested for cyanide with ferrous sulphate, ferric chloride and hydrochloric acid. No prussian blue precipitate was obtained and therefore *no organic nitrogen* was present.

Twenty days after the above quantitative tests the liquid still being pumped up was analysed again:

(2) *Estimation of sugars by reduction of Fehling's solution:*

Before inversion	After inversion	Hexose	Cane sugar
0.034 per cent.	0.036 per cent.	0.034 per cent.	0.0019 per cent.

Total residue on evaporating to dryness: 0.14 per cent.

Organic residue 0.08 per cent.

Inorganic „ 0.06 „

This result is interesting if compared with the results of the analysis of Fraction I. It will be seen that the amount of organic matter has fallen off considerably. This is what would be expected, as the supply of storage carbohydrates in the parenchyma surrounding the vessels will have been very much depleted. The organic residue obtained, however, as in the previous case, is larger than the figure for sugar obtained by the reduction of Fehling's. It is also interesting to note that the amount of inorganic matter is practically the same as before. As the amount of liquid exuded by the root gradually diminishes, the organic substances present also decrease, whereas the inorganic remain constant, from which it would seem that the amount of liquid passed into the xylem from the surrounding tissue was dependent to a much greater extent upon the concentration of organic than of inorganic solutes in the sap.

Kastle and Elvove (15) had found *nitrite* present in the liquid collected by them in root pressure experiments with *Fuchsia*, an observation we had confirmed, so that the liquid from the vine was tested for nitrite with acidified solution of potassium iodide and starch. The sterile liquid gave a very strong reaction for nitrite. In the liquid which had not been kept under toluol no nitrite was found, but *nitrate*, showing that oxidation was proceeding, doubtless due to the presence of bacteria.

Experiments with Fuchsia

The plants were cut down about an inch above the level of the soil, and a piece of glass quill tubing attached by means of pressure tubing which was securely wired to the cut stump. The liquid exuded into the glass tubing was collected and examined, but in this case no evidence could be obtained of the presence of sugars—not even with *Molisch's test* (Plimmer (19), *loc. cit.* p. 193), a very sensitive reaction given by all sugars.

Total residue on evaporating to dryness = 0.16 per cent.

Organic residue 0.07 per cent.

Inorganic ,, 0.09 ,,

It will be seen from this that although no evidence could be obtained for the presence of sugars, yet there is an organic solute (or solutes) present in the sap.

We have been able to throw very little light on the additional organic substances present in these experiments with both Vine and

Fuchsia. Our first thought was organic acid, but tested with the usual series of indicators (Cole⁽⁶⁾, *loc. cit.* p. 22) the P_H value of the sap in the vines was practically that of true neutrality. No calcium salts could be precipitated from the concentrated juice, so that in particular any soluble salts of oxalic acid seemed to be absent. This was a surprise to us because parallel experiments in progress in the laboratory by Miss L. Woffenden had drawn attention to the fact that the hydathodes of Fuchsia gradually become blocked with an amorphous deposit which behaves like calcium oxalate; and that in the neighbourhood of the hydathodes of many plants, crystalline deposits of calcium oxalate are abundant.

An arrangement was devised by which the liquid from the vines, after concentration under reduced pressure, could be continuously extracted by ether. The ether was purified by redistillation after standing over stick potash for several days, a precaution that experience proved essential. From the ether extract, on evaporating, a substance separated out either in thin plates or needles with serrated margins. The substance crystallised best from chloroform. This substance is readily soluble in warm ether, sparingly soluble in cold, and has a slightly pungent odour faintly reminiscent of condensation products of formaldehyde. This fact led us to apply Rimini's test as modified by Schryver⁽²³⁾ with the result that the substance, whilst giving no reaction for free formaldehyde, gave a very strong reaction for formaldehyde in combination. We are unable at present to give any clearer indication of the nature of this substance and indeed should be very grateful for suggestions. In any case it cannot be the only other organic compound present besides sugar, as from 800 c.c. of the original sap, concentrated under reduced pressure and then continuously extracted with ether, a yield of only 0.08 gm. was obtained. As some 0.06 per cent. of organic substance has to be accounted for in the vine sap, obviously some other compounds are present.

Solutes in sap exuded by stems or leafy branches

Liquid exuded by the activity of stems or leafy twigs under the conditions described in a preceding section (p. 64) may also be expected to contain organic substances, but as the quantity of sap obtained in any particular experiment is not large, it is not possible to give very precise data as to their nature or quantity. Very varying results were obtained in the long series of experiments conducted under this head, and they are probably not worth detailed analysis.

In some cases no indication of sugar was obtained, even by Molisch's reaction, but usually this test gave clear indication of the presence of sugar. Sometimes a marked reduction of Fehling's solution took place immediately, more frequently no reduction with Fehling's was obtained until after inversion with acid and subsequent neutralisation, indicating that in these cases cane sugar was the only sugar present. In other cases the formation of glucosazone gave indubitable evidence of a hexose sugar; the solutions were too dilute or too opalescent to enable these indications to be extended by the use of a polarimeter. In spite of the opalescence frequently noted in the liquid, the bi-uret test never gave indication of the presence of a protein or peptone in the solution.

The osmotic concentration of the sap was obtained in some cases by Barger's method. (See Barger⁽³⁾ and Yamakami⁽²⁴⁾. This method has already been used in plant physiological work by Halket⁽¹⁴⁾.) The most concentrated saps examined were found to be isotonic with 0.25 *M* sodium chloride solution.

IV. EXCHANGE OF SUGAR BETWEEN THE XYLEM AND THE SURROUNDING PROTOPLASTS

During the course of this work, Flood's paper⁽¹³⁾ appeared upon the nature of the liquid issuing from the hydathodes of *Colocasia antiquorum* (Schott). The liquid issues through the hydathode when the sap in the xylem is under positive pressure. Flood's investigations show clearly that it passes through no filtering mechanism in its transit through the hydathode and yet the water that issues is as pure as conductivity water. How can this statement be reconciled with the statements and experimental evidence referred to above, showing that the xylem sap contains sugar or other organic solute? The reconciliation is not difficult and has already been indicated; see, for instance, Ewart⁽¹²⁾ (*loc. cit.* pp. 349–351, *Phil. Trans.* 199 B), where cogent experimental evidence is given for the absorption of solutes in their passage along the xylem vessel by the process of *adsorption*.

In the course of our own work we endeavoured to demonstrate quantitatively the removal of sugar from the stream of water passing along the xylem. These experiments are perhaps more open to criticism than those of Ewart, as it is very difficult to obtain quantitative results on this point which will have the precision of the qualitative demonstrations given by Ewart.

The basis of our method was to suck sugar solution or distilled water through a long piece of stem, on which all lateral branches had been cut off and the surfaces thus exposed sealed with wax. The change of concentration in the liquid after its passage through the stem was then noted. The method suffers from many disadvantages. When a liquid is drawn through a long piece of stem in this way some is invariably lost in transit, and on the other hand the first few drops withdrawn consist of liquid previously within the xylem vessels. Furthermore, a certain amount of sugar will diffuse from wounded cells at the end of the stem into the liquid being collected. An actual experiment showed that the latter source of error could be neglected, the amount being negligible in comparison with the quantities of sugar drawn through the xylem; and the other sources of error are not sufficient to vitiate the few conclusions that have been drawn from the results obtained.

Figures obtained in typical experiments are now given. Table I shows the difference between the amounts of sugar absorbed during passage through living and dead stems respectively. These experiments probably indicate the qualitative difference that exists between physical adsorption of sugar by the walls of the containing tissue and the physiological absorption which reinforces that process in the living stem.

TABLE I

Plant	Solution	Mercury manometer cms.	Time hrs.	Amount liquid through c.c.	Amount liquid lost c.c.	Original sugar %	After passage %	Absorbed %	Absolute amount sugar absorbed gm.
{ Sycamore living	Mixture of cane sugar and glucose	35-49	22	64	1	3.4	1.0	2.4	1.54
{ Sycamore dead	Glucose	66-70	18½	86	34	2.0	1.5	0.5	0.4
{ Elm living	"	65	22½	145	20	1.9	1.4	0.5	0.6
{ Elm dead	"	60-66	22½	46	14	2.0	1.8	0.2	0.09
{ Syringa living	"	72	24	9	3	2.0	.63	1.37	0.123
{ Syringa dead	"	72	23	4	6	2.0	1.7	0.3	0.012

If the removal of sugar from the dead stem is an adsorption process then it should be reversible. The following data for another experiment show that, within the limits of accuracy which the experimental conditions permit, the process seems to be reversible.

Stem of *Syringa vulgaris* L. killed by steam.

Irrigated with distilled water			
	Hexose sugar %	Absolute amount gm.	
First fraction collected	0.4	0.03 (released by stem)	
Second fraction collected	0.15	0.02 ,, ,,	
Irrigated with 2 % glucose			
	1.5	0.6 (retained in stem)	
Irrigated with distilled water again.			
First fraction 	1.2	0.2 (released by stem)	
Second ,, ...	1.0	0.12 ,, ,,	
Third ,, ...	0.3	0.01 ,, ,,	

In no case was there any increase in reducing power after inversion.

But physiological absorption represents a process of diffusion through a plasma membrane and this again may be expected to be a reversible process and dependent upon the concentration of the sap within and without the protoplast.

The data given in Table II show that when distilled water is drawn through living stems sugar is released into the ascending stream and that if a current of sugar solution be substituted then the reverse action takes place and a certain proportion of the sugar is removed in passage.

TABLE II

Stems (of *Æsculus hippocastanum* L.) irrigated with water

Condition	Mercury mano- meter cms.	Duration of irriga- tion hrs.	Volume liquid through c.c.	Volume lost c.c.	Hexose after irrigation %	Change in concen- tration %	Absolute gain or loss hexose gm.
I. Buds opened	58	9	37	38	0.1	+0.1	+0.04
II. In leaf	50	8½	175	35	0.0006* (practically nil)	+0.0006*	+0.001*

Same stems irrigated subsequently with 2 % glucose solution

I. Buds opened	70	5½	31	9	0.16	-1.84	-0.57
II. In leaf	70	4½	41	9	0.56	-1.44	-0.59

* This liquid after inversion showed 0.04 % cane sugar present or 0.07 gm. absolute amount.

These data suggested that if it was desired to state quantitatively the conditions existing in the vascular strand at any particular time with reference to the absorption or release of sugar, this could best be done by finding approximately the concentration of sugar solution with which the protoplasts of the surrounding tissues seem to be in equilibrium. This concentration of sugar should suffer neither appreciable loss nor increase during its passage through the stem. In

Table III values are given for the change in sugar content in two successive fractions of liquid drawn through the same stem, and in the cases numbered 3, 4 and 5 this equilibrium condition is approximately reached.

TABLE III

Stems of *Acer pseudoplatanus* L. irrigated, liquid collected in two successive fractions A and B

Condition	Irrigating solution	Duration irri- gation hrs.	Volume liquid through c.c.	Volume liquid lost c.c.	Cane sugar after irri- gation %	Absolute amount cane sugar gm.	Gain or loss	
							%	Absolute amount gm.
1. In bud	Distilled water	{ A 2 B 1½	62 48	10 —	0.12* 0.025	0.07 0.001	+0.12* +0.025	+0.07* +0.001
2. In bud	0.17 % cane sugar	{ A 2½ B 1½	25 20	25 —	0.12 0.095	0.03 0.019	-0.05 -0.075	-0.013 -0.015
3. In bud	0.17 % cane sugar	{ A 4½ B 16½	3 3	14 —	0.15 0.15	0.005 0.005	-0.02 -0.02	-0.0006 -0.0006
4. Leaves just out	0.17 % cane sugar	{ A 2½ B 2½	73 22	4 —	0.24 0.12	0.18 0.03	+0.07 -0.05	+0.05 -0.01
5. Leaves just out	0.16 % cane sugar	{ A 2½ B 2½	12 15	8 —	0.20 0.11	0.024 0.02	+0.04 -0.05	+0.005 -0.008

* In this one case a slight trace of hexose sugars was detected in the liquid.

This conception could be utilised to analyse the differences in sap between trees in different developmental stages, for instance, trees in bud, with buds bursting, in leaf, etc. Dixon and Atkins(1) (*loc. cit.* p. 195) have found that the sap ascending in the xylem is most concentrated just as the leaf buds are bursting. The experiments recorded in Table III also support this conclusion, thus numbers 4 and 5, where the leaves are just opening, are the only stems giving out sugar into the sugar solution passed through them.

Curtis(8) has recently described some experiments in which he has ringed the phloem at two levels on the same stem and studied the subsequent fate of the starch reserves between these two rings. He regards this observation as strong evidence for his view, elaborated in this and in a subsequent paper(9), that the xylem stream is not responsible for any appreciable transfer of carbohydrates from root to stem or from one region of the stem to another. Evidence obtained in this manner, however, could not destroy the cogency of such evidence as that summarised by Atkin or that presented above, which deals with the analysis of sap moving in the xylem strands. It is also clear that the non-removal of starch from the region between the two rings in Curtis' experiments might be due to the fact that the sap traversing the xylem between the two rings was in

carbohydrate equilibrium with the starch storing tissues in this region.

DISCUSSION

From a consideration of the foregoing observations and experiments there emerges a definite view of the part played by exudation in regulating the flow of sap in the plant. The basal conception is that of an aqueous solution moving within a readily permeable xylem tube and able to diffuse outwards laterally along the permeable walls of the neighbouring protoplasts.

At any moment certain protoplasts near the xylem tube have specially permeable plasma membranes and thus permit the exchange of organic substances between their sap and the solution in the xylem tube. The direction in which the solute would pass depends upon its relative concentration in xylem vessel and protoplast so that in one region the liquid in the xylem vessel may be receiving the substance from the surrounding cells, in another region the same solute may be diffusing away from the xylem into the neighbouring tissue. Thus we arrive at the conception of an equilibrium concentration for any solute, which is illustrated experimentally by cases where sugar solution passes through the vascular tracts of a living stem without appreciable gain or loss of concentration in passage.

It is the presence of these organic solutes in the xylem sap which maintains the osmotic pressure of the sap at a level permitting the manifestation of exudation pressures when the surrounding parenchymatous tissues are in contact with a more dilute aqueous solution. In the root these exudation pressures may be vigorous and well maintained because the lateral leakage of these solutes from the xylem tube is so efficiently prevented by the endodermis. In stem and leaf exudation pressures may be temporarily demonstrated under suitable conditions, but the absence of a functional endodermis permits too rapid a leakage of the necessary solutes from the xylem for the pressures to be long maintained.

The water column in the xylem is usually ascending in a leafy plant under the pull exerted as the result of transpiration from the leaves, and exudation pressure is a relatively unimportant phenomenon. Neglect of this consideration has possibly led to the attitude of opposition in which theories of the mechanism of exudation pressure appear to find themselves, in relation to the admirably clear case for the mechanism of transpiration suction presented by Dixon (10) and his co-workers. Thus in the brief preliminary account of her work given by the late Sarah Baker (2), after drawing attention

to the fact that Dixon and Joly(11) in their experiments on the cohesive force of liquids had neglected to take account of temperature effects, she proposed *an alternative theory* of the ascent of sap based on a mechanism invoking exudation pressures in the root. But no theory based on exudation pressures in root or stem can replace Dixon and Joly's hypothesis based on the consequences of transpiration from the leaf. So long as the sap is ascending under negative pressure it must be driven by a force exerted from the highest point it reaches—the leaf.

However, in some plants at all times (as in submerged water plants) and in many plants at some times (as in deciduous trees in the spring) ample scope can be found for the utility of exudation pressures whilst observation and experiment supply full evidence of their existence. Reinders(21) has published a most clear and interesting discussion of the relation between the transpiration hypothesis of sap ascent and the hypotheses that assume the assistance of exudation pressures along the path of the ascending water column. He points out, as Copeland(7) had previously done, that the outstanding difficulty in the way of fully accepting the cohesion transpiration hypothesis, was the failure of its exponents to deal with the lack of experimental demonstration of the gradient of pressure in the path of the water column, that must be present if the column was entirely raised by the action of forces exerted in the leaves.

In some interesting preliminary experiments Reinders showed that if a series of manometers were placed upon cut branches at different heights of the same tree, the pressures recorded were quite without reference to the vertical position of the manometer in the series, but that if the tract of stem was killed that was bearing the branches with the manometer, the pressure readings then recorded a suction gradually decreasing from the upper region towards the base of the stem. These experiments retain their suggestiveness, although further experiment has led Reinders(22) to realise the great experimental difficulties that lie in the way of obtaining significant records of intermediate pressures in the sap column from the readings of such manometers.

Reinders' experiments seem to us to provide valid support for the position taken up in this paper that whilst the transpiration of the leaves remains the main force actuating the ascent of water in freely transpiring land plants, yet exudation pressures due to parenchymatous tissues associated with the xylem tract, are always potentially possible.

SUMMARY

In this paper attention has been confined to the nature and conditions of the exchange of solutes between the xylem and the parenchyma around the vascular strand. Experimental grounds are given for the following conclusions:

(1) At any moment certain cells around the vascular strand and within the vascular cylinder have especially permeable protoplasts; in some cases these protoplasts would seem to be more readily permeable by acid or basic ions. This phenomenon may account for the continued presence of solutes within the xylem vessel during the manifestation of exudation pressure.

(2) Given suitable experimental conditions, exudation pressures can be exhibited by stems and leaves as well as by roots. In this connection some of the earlier work by Pitra, Kraus and Boehm is reviewed.

(3) In the case of *Fuchsia*, failure to obtain the evidence of exudation pressure in the case of leafy twigs, can be traced to leakage from the hydathodes at the vein ends. If these exits from the veins are blocked, exudation pressures can easily be demonstrated under suitable conditions.

(4) The solutes present in the xylem sap are both organic and inorganic. As the organic solutes fell off in quantity in the sap rising from a cut vine, the exudation pressure began to fail whilst the supply of inorganic solutes still remained approximately constant. Organic solutes would thus seem to be more directly responsible for the osmotic pressure effective in causing the flow of sap. On theoretical grounds this result would be expected as the inorganic solutes present have probably diffused across the endodermal barrier.

(5) The organic solutes in the case of the vine were mainly sugars, disaccharides and hexoses, but other organic substances were present, and one of these could be extracted by hot ether and subsequently obtained crystalline. Amongst the inorganic solutes, the presence of nitrites previously reported was confirmed, nitrites being found both in *Vine* and *Fuchsia*.

(6) The fact that the water dropping from the hydathodes of *Colocasia antiquorum* (Schott) is practically free from solute, can readily be explained as due to the absorption of the solutes during the upward passage of the sap. This absorption appears to involve both physical adsorption and physiological absorption, and both processes are reversible.

(7) The conception is developed of an equilibrium concentration for a sugar solution which would neither lose nor gain sugar during

its passage through the xylem. This equilibrium concentration would vary in the same plant at different seasons.

In the tree stems investigated the evidence obtained agreed with the conclusion of Dixon and Joly that the highest value for the equilibrium concentration would be found in the xylem at the time that the leaf buds were bursting.

BOTANICAL DEPARTMENT,
THE UNIVERSITY, LEEDS.

REFERENCES

- (1) ATKINS, W. R. G. *Some Recent Researches in Plant Physiology*. Whitaker and Co., 1916.
- (2) BAKER, SARAH. Liquid Pressure Theory of Circulation of Sap in Plants. *Brit. Assoc. Report*, Manchester Meeting, p. 722. 1915.
- (3) BARGER, S. A microscopic method of determining molecular weights. *Journal Chem. Society*, **85**. 1906.
- (4) BOEHM, JOSEF. Ueber Druckkräfte in Stammorganen. *Bot. Zeitung*, 38 Jahrgang, p. 33. 1880.
- (5) CLOWES and COLEMAN. *Quantitative Analysis*. S. and A. Churchill.
- (6) COLE, S. W. *Practical Physiological Chemistry*. W. Heffer and Sons
- (7) COPELAND. The rise of the Transpiration Stream. *Bot. Gaz.* pp. 161 and 260. 1902.
- (8) CURTIS, OTIS F. The Upward Translocation of Foods in Woody Plants. I. *Amer. Journal Bot.* **7**, pp. 101-124. 1920.
- (9) ——— The Upward Translocation of Foods in Woody Plants. II. *Amer. Journal Bot.* **7**, pp. 286-295. 1920.
- (10) DIXON, H. H. *Transpiration and the Ascent of Sap in Plants*. Macmillan and Co., 1914.
- (11) DIXON, H. H. and JOLY. On the Ascent of Sap. *Ann. Bot.* **8**, pp. 468-470. 1894. And *Trans. Roy. Soc.* **186**, B. I, pp. 563-576. 1895.
- (12) EWART, A. J. On the Ascent of Water in Trees. *Phil. Trans. Roy. Soc.* **198** B. p. 41. 1905; and **199** B. p. 341. 1908.
- (13) FLOOD, MARGARET. Exudation of Water by *Colocasia antiquorum*. *Procgs of the Royal Dublin Society*, **15** (N.S.), No. 36. April, 1919.
- (14) HALKET, ANN C. On Methods of Determining Osmotic Pressures. *New Phyt.* **12**, p. 164. 1913.
- (15) KASTLE and ELVOVE. The Reduction of Nitrates by certain Plant Extracts. *Amer. Chem. Journal*, **31**, p. 606. 1904.
- (16) KRAUS, C. Untersuchungen über den Säftedruck der Pflanzen. *Flora*, **82**, 65 Jahrg. N.R. 40, pp. 2, 17, 49, 105, 145, 277, 419, 435, 520 and 565. Also *Flora*, **83**, 66 Jahrg. N.R. 41, pp. 2, 25, 81 and 129.
- (17) OSTERHOUT, W. J. V. Some Quantitative Researches on the Permeability of Plant Cells. *The Plant World*, **16**, pp. 129-144. 1913.
- (18) PITRA, AD. *Jahrb. für Wiss. Bot.* **11**, p. 437. 1878.
- (19) PLIMMER, R. H. A. *Practical Organic and Biochemistry*. London, 1918.
- (20) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phyt.* **19**, p. 198. 1920.
- (21) REINDERS, E. Sap-raising forces in living wood. *Proceedings Koninklijke Akad. van Wetenschappen te Amsterdam*, pp. 563-573. 1910.
- (22) ——— Das Manometer in der Saftsteigungsfrage. *Extrait du Recueil des Trav. bot. Néerlandais*, **10**, pp. 1-66. 1913.
- (23) SCHRYVER, S. B. The Photochemical Formation of Formaldelyde in Green Plants. *Proc. Roy. Soc.* **82** B, pp. 226-232. 1909-10.
- (24) YAMAKAMI, K. Barger's microscopical method of determining Molecular Weights (Part I). *Biochemical Journal*, **14**, pp. 103-113. 1920.

A CRITICAL STUDY OF CERTAIN UNICELLULAR CYANOPHYCEÆ FROM THE POINT OF VIEW OF THEIR EVOLUTION

By W. B. CROW, M.Sc., F.L.S.

(University College of South Wales and Monmouthshire)

(With 1 figure in the text)

UNLIKE many of the groups of unicellular organisms the class Cyanophyceæ, Myxophyceæ or Blue-green Algæ seems remarkably well defined. Whilst no two authors are agreed, for instance, as to the limits of that vague assemblage of motile types generally known as Flagellata, yet a review of the literature shows that modern systematists are in harmony in their definition of the Cyanophyceæ. The systems of classification proposed for the group by Forti(7), Tilden(16), Lemmermann(14), Bessey(2), West(18), and Kirchner(11) not only agree in containing the same genera but also recognise the same families. It is true that Lemmermann divides the Nostocaceæ, whilst according to Bessey certain genera of Bacteria should be admitted. But the system remains essentially the same. The only other modification that has been proposed in recent years is the exclusion of *Porphyridium* from the Chroococcaceæ since this genus was placed by Brand in the Bangiales. This, at first sight an unimportant change, is of considerable significance, for it raises the question whether other supposed Chroococcaceæ may not be reduced members of this Red Algal series or of related groups. Whatever views may be held with regard to the Rhodophycean origin of *Porphyridium*¹, as will appear subsequently, many unicellular Cyanophyceæ are best regarded as relatively primitive forms, bearing a somewhat similar relation to the filamentous Cyanophyceæ as the Tetrasporales to the filamentous Chlorophyceæ.

It is not necessary to enumerate here the many characters which are common to all the Cyanophyceæ and which distinguish them so sharply from all other Thallophyta. A great deal of attention has been paid to these common characters; the less striking differences which exist between the species and genera have been almost neglected except by purely taxonomic writers and have not been treated

¹ See Staehelin, *Ber. d. deutsch. bot. Ges.* 1914; Kuffenrath, *Inst. bot. Léo Errera*, 1920. But also Brand, *Berichte*, 1917.

from an evolutionary point of view. Some knowledge of the phylogenetic relationships of the various forms is necessary to a clear understanding of the origin of the group as a whole and hence of the Protista in general. In these pages the characters of certain of the unicellular forms, belonging to the family Chroococcaceæ, are analysed, with a view to ascertaining their importance in the evolution of the group, and hence in helping to establish a satisfactory system of classification. Now the characters used by systematists to distinguish the various members of the Cyanophyceæ, particularly the unicellular types, are perforce of the nature of very minute differences, since these organisms show little morphological variety. The true value of the characters employed can only be judged by a study of the variation exhibited among individuals of the various species, more particularly such as occur under natural conditions, and by an examination of the possible underlying factors whether internal or external.

CELL-FORM

The family Chroococcaceæ includes the simplest known representatives of the Cyanophyceæ. In its most elementary state the organism of such a genus as *Chroococcus* or *Glæocapsa* consists of a single spherical cell, the form here owing its origin to purely physical factors. It is, in fact, the same as that of any inert amorphous fluid body placed in a medium with which it is immiscible. This is the dominant form of the cell in the Chroococcaceæ, and it should be noted that not only is it to be considered as the primitive one, but that there would be a tendency for cells which have taken on other forms in response to stimuli, whether of external or internal origin, to revert to the original spherical state when the disturbing factors are removed. Spherical organisms are not uncommon, and their form by itself by no means proves a primitive position in the scale of evolution. One of the commonest modifications seen in the Chroococcaceæ is found in the polyhedral form taken on by the cells of many species as a result of mutual pressure where the cells are associated in groups. In many species of *Chroococcus* the angular character is seen to be directly correlated with the degree of crowding of the cells, those in the less dense colonies or parts of colonies being perfectly spherical. From the point of view of evolution, then, such changes of cell-form as are met with in certain individuals of species of *Chroococcus* are of no great significance. The degree of crowding of the cells depends on the rate of cell-division and the rate of mucilage secretion, two factors which, as will be shown later, are very

variable and presumably depend to a large extent on external conditions. But in some *Chroococcaceæ* the angular form of the cell cannot be explained so readily. Throughout the genus *Tetrapedia* the cells show various polyhedral shapes, in fact it is the remarkable cell-form that alone affords a satisfactory distinction from the allied *Merismopedia*. There is no evidence that the angular form is due to compression, since the individual cells are often free or very loosely associated. It is in fact an inherent quality of the cell and, under natural conditions at least, is retained from one generation to the next. Of course the angular character may have taken origin in relation to a former crowded type of colony. In some species of *Merismopedia* mutual pressure of the cells gives them an angular character just as in *Chroococcus*, except that they are quadrate in the former case, in correspondence with the mode of cell-division in *Merismopedia*. But in *Tetrapedia* there are other complications which are not explained on this view. For the cells are compressed in a plane at right angles to their division plane, and moreover they possess a very remarkable pair of constrictions dividing the cell at right angles into four parts. The first of these features is primarily due to the differential growth of the cell, which enlarges chiefly in directions at right angles to the directions of cell-division. The second feature is also related to the planes of fission. The position of the constrictions coincides with these planes, and in fact the partial segmentation of the cell-body in this way is little more than the precocious separation of daughter cells from the protoplast. The origin of the character may be traced in the genus *Holopedium* in which one species (*H. Dieteli* Richt.) differs from its allies inasmuch as the cells become more or less lengthened as seen from the surface of the colony before division and are recorded as being slightly constricted in the middle. The lack of this constriction in the other species and in the genus *Merismopedia* merely indicates that in these forms division is more direct. The constricted form of cell is also incipiently developed in *Synechococcus*, where, however, there is only one plane of division and consequently only two lobes. In *S. ulcericola* Rabenh. the hour-glass shape of the cells is a constant feature from the moment of their origin.

A more common departure from the spherical cell-type is seen in those forms which have elongated cells. Where this is the case we may speak of a definite polarity, indicated by a preferential growth in one direction. The phenomenon is related to the process of cell-division, the axis of elongation being at right angles to the plane of

fission. It is absent in many forms. Thus in some species of *Chroococcus*, of *Glæocapsa* and of *Merismopedia* the mature cells are spherical and the products of cell-division, when they first appear as separate individuals, *i.e.* on completion of the fissure between them, are hemispherical. But in other species of these genera (*e.g.* *Chr. caldariorum* Hansg., *M. elegans* A. Br., *Gl. caldariorum* Rabenh.) the cells often attain a length equal to about twice their width before dividing, so that the daughter cells are approximately isodiametric when first formed. In still other Chroococcaceæ (*e.g.* *Aphanothece* spp.) the individual cells are cylindrical from the moment of their formation. Since, as has been stated already, cell-elongation is related to the process of cell-division, the production of cylindrical daughter cells chiefly occurs in genera where successive planes of cell-division are parallel. Here the direction of elongation becomes a fixed axis.

When amount of growth is great in proportion to the number of cell-divisions the cells may reach a considerable length (*e.g.* five to ten times their diameter). In the longer forms of *Aphanothece* and *Glæothece* the cells generally become curved. The curvature seems to be a direct effect of the greater length. For out of thirty-seven species of *Aphanothece* and *Glæothece* listed in De Toni's *Sylloge Algarum* six are described as having cells attaining a length greater than three times their diameter, and in all of these the cell may be curved. But of the thirty-one short-celled types only *Glæothece lunata* W. et G. S. West shows curvature. Examination of collections of individual cells of long-celled species of *Aphanothece* also shows that curvature is correlated with length, although in dealing with a single species this is not so obvious since a wide range of variation is not covered. It would seem, then, that curvature of the cell is not of any morphological significance. With bodies of protoplasm unprovided with any special mechanical support it would, in fact, be surprising if the more elongated forms did not frequently exhibit bending.

In the foregoing we have spoken of long-celled species and short-celled species. For if collections of cells of any one species of *Aphanothece*, for instance, be taken from one and the same habitat, it is found on examination that little or no variation in length occurs other than such as may be due directly to diversity in the age of the individual cells. It is therefore probable that degree of elongation would be of value in classification, if different species were compared under similar conditions of growth.

Before leaving the elongated cell-type reference must be made to

the spindle-shape of *Dactylococcopsis*. The tapering extremities of the cell are a very constant feature of this genus, as of many species of the Oscillatoriaceæ. In most species of *Dactylococcopsis* the cells show a tendency towards spiral coiling, but in the peculiar planktonic *D. acicularis* Lemm. the cell is straight and needle-like. The stiff character of the cells, which is seen to a less extent in other species of *Dactylococcopsis* affords a very clear systematic distinction from the *Aphanothece* type. In its cell-form *Dactylococcopsis* connects the Chroococcaceæ with certain of the Oscillatoriaceæ. By a slight elongation of the spiral which we may imagine as forming the axis of the cell we pass on to forms belonging to the genus *Spirulina*. These have a unicellular spiral filament¹. It is worthy of note that there are no straight forms corresponding with the genus *Spirulina*. Here again the spiral form may be due to mechanical factors. The species are all characterised by rather slender cells, an average taken from the recorded measurements of seventeen well-defined species gives the diameter of the trichome as 2.0μ . Except in size there appears to be no essential difference between *Spirulina* and *Arthrospira*, although the latter always has septate filaments. For an average of the width of trichome recorded for the seven recognised species of *Arthrospira* is found to be 5.1μ and this suggests that the septate condition in *Arthrospira* may have been developed in relation to the more robust character of the plant. Where the septate condition obtains, *i.e.* in the majority of the Oscillatoriaceæ, the straight form of the filament becomes mechanically efficient and is, of course, the predominant type in the family. There are reasons for supposing that the unbranched trichome found in many Oscillatoriaceæ is homologous with the single cell of the Chroococcaceæ. The observations of Fischer(6), who found that the pigment-bearing region of the cell in *Oscillatoria* and *Lyngbya* has the form of a cylinder with open ends, so that, apart from the transverse septa the central region forms a continuous core throughout the trichome, support this view. The work of Fritsch(8) on the structure of the membrane, showing that in *Oscillatoria* the sheath is not split on the formation of a new transverse septum, is also in harmony with it, although this investigator suggests that the constrictions of the sheath opposite the septa in some species indicate a compound origin. These constrictions, however, were probably developed in the first place by the lower rate of mucilage secretion at the region of the transverse septa. At these

¹ Traces of septa may be observed in some species on staining by special methods, see Zuelzer, *Archiv f. Protistenkunde*, Bd. 28, 1911.

regions the activities of the cell are largely directed towards the formation of this internal membrane and less active mucilage secretion would occur externally in these zones. Similar constrictions occur between the coenocytes of many Siphonocladiales.

Returning to the modifications of cell-form in the Chroococcaceæ themselves, it may be noted that the elongated type may sometimes be best explained as due to mutual pressure of the cells of a colony. Thus an examination of the tabular colonies of *Merismopedia* (e.g. *M. elegans* A. Br.) from their edge, shows that the cells are elongated at right angles to the surface of the whole group. Cell-division does not occur in this direction and it is easy to relate the elongated form to pressure rather than to polarised growth. Cell-elongation of the kind indicated here reaches its maximum development in the genus *Holopedium*.

The pear-shaped or heart-shaped cells of *Gomphosphæria* represent another distinctive type. In these cells we can speak of a distinct base and apex and hence the genus must be relegated to the Chamæsiphonaceæ. The fact that the stalks at the base of the cells are joined to one another instead of to a substratum, thus giving the plant a free floating habit, has obscured the true affinities of the genus. The common occurrence of "gonidia" in *Gomphosphæria* verifies this view and thus places it outside the scope of the present investigation.

CELL-SIZE

When material of a single species from a single habitat is dealt with, no great variability in the character of cell-form is encountered. The character of cell-size, however, appears to be somewhat more plastic. Table I gives some data obtained by actual measurement of collections of various species for the character in question. It was not thought necessary to take more than one hundred individuals, since the number of classes into which the variates could be arranged was limited by the means of measurement. Table II gives the recorded diameter of the protoplast for a number of well-defined species and such as are easily recognisable apart from the size of cell. These tables show that there is an appreciable variability. In the case of some of the species of *Glæocapsa* and of *Chroococcus* in Table I such variability may indicate the existence of several distinct races morphologically indistinguishable. But variation is also appreciable in the cell-size of *Cælosphærium*, *Microcystis* and *Merismopedia* in the table, where the protoplasts measured in each collection were members of one and the same colony, so that here we are dealing with

pure lines. The same applies to the species symbiotic in lichens, which were chosen for this reason, the members of each collection being measured from one and the same lichen thallus¹. In such cases it will be seen that the variability, although actually small, is not altogether insignificant when compared with the actual dimensions of the cell. Nevertheless, as will appear in comparison with other characters to be dealt with subsequently, cell-size is one of the more constant specific characters in the Chroococcaceæ.

It will also be seen from the tables, especially from Table II, that the variability differs greatly among the different species. At first it might be thought that specific variability would be a fundamental property of the various species and useful as a criterion in classification; further, that greater variability in certain species of *Chroococcus* indicated a more primitive position for these forms in the scale of evolution, since in general the more primitive members of a group show greater variability. But variation from the mean depends on the reaction or reactions caused by various stimuli, either external or internal to the cell. It has been shown by such work as that of Brand (3) on *Glæocapsa* and of Brunnthaler (4) on *Glæothece* that organisms such as those we are dealing with, are, like most other simple forms, very sensitive to external stimuli, *e.g.* those of light and osmotic or chemical conditions. Hence it must be assumed that such extrinsic factors play the chief part in bringing about variation. Now the action of these stimuli will be to some extent dependent on the size of the cell. Thus cells of small mass will be acted upon to a less extent by certain chemical and other stimuli than those of greater mass, whilst those of small surface will respond less to photic, ionic and osmotic stimuli than will those of greater surface². Hence there is no indication that the rather remarkable variability of some of the species tabulated in II is anything more than the effect of their larger cell-size.

INTERNAL STRUCTURE OF THE CELL

A few facts regarding the minute internal structure of the Cyanophycean cell are of interest from the present point of view, although the unicellular forms have been less studied than the filamentous ones and the whole subject must be approached with diffidence, owing to the very diverse conclusions reached by those who have investigated it. Most writers are agreed, however, and it can easily

¹ I have to thank Prof. Darbishire for the loan of specimens of lichens.

² It is here merely assumed that living cells are influenced by the ordinary physical laws of mass and surface action.

be demonstrated in the living cell of many forms, that there are three parts, namely the central region, rich in central granules, the peripheral protoplasm containing the pigment and cyanophycin granules, and the cell-membrane. In both the filamentous and unicellular types some species having these parts clearly differentiated, and other species where they are not so distinct, occur. Chodat(5) has recorded a more or less definite central region for cells of *Chroococcus turgidus* (Kuetz.) Naeg. Olive(15) and Gardner(9) described such a region as occurring in species of *Glæocapsa*. Some of the Chroococcaceæ have been more recently investigated by Miss Acton(1) who found that a definite central region was differentiated in the cells of *Chroococcus macrococcus* (Kuetz.) Rabenh., *Merismopedia elegans* A. Br. and *Aphanothece prasina* A. Br., whilst in those of *Merismopedia glauca* (Ehrb.) Naeg. and a species of *Glæocapsa* no such specialised portions of the protoplast appeared; *Chroococcus turgidus* (Kuetz.) Naeg. and others showed an intermediate condition. Finally, in the genus *Myxobactron*, according to West(18), there is no central region.

The present writer, working with material fixed in formalin, has found a very definite central region in the protoplasts of *Microcystis flos-aquæ* (Wittr.) Kirchn. and *Merismopedia elegans* A. Br., staining with the fuchsin or methylene blue methods described by Kohl(12). The deeply staining material of this central region varied in arrangement in different individuals, but it is a significant fact that the differentiation of this region was found to be constant for a large number of cells examined in both species. It is possible therefore that the degree of development of the central body might afford some guide to the classification of the Chroococcaceæ, although further work is of course very essential in this connection.

Of the remaining cytological characters many are very dependent on external influences(12) and cannot be brought to bear on the present problem. Such are the presence or absence of cyanophycin granules, central granules, glucoprotein, glycogen, sugar and fatty oils. All these substances, too, are widespread in the Cyanophyceæ and hence differential chemical characters are not easy to establish. The presence or absence of pseudovacuoles has been used by Lemmermann(14) to distinguish various species of *Microcystis* and *Cælosphærium*. He finds that the species of the former genus fall into two biological groups. The first consists of those practically confined to the plankton, often forming "water-bloom" and containing pseudovacuoles in their cells (e.g. *M. æruginosa* Kuetz., *M. flos-aquæ* (Wittr.) Kirchner), the second of those which are without pseudo-

vacuoles and which may occur both in plankton and benthon (*M. pulverea* (Wood) Migula, *M. fuscolutea* (Hansg.) Migula). A transition between the two groups is found in *M. ochracea* (Brand) Lemm. and *M. elabens* (Meneg.) Kuetz., these being at first benthonic and without pseudovacuoles, later developing these structures and becoming planktonic. The important feature is that under given ecological conditions, *e.g.* in freshwater plankton, they are fairly constant for the species. An examination of a large quantity of material of several species of *Microcystis* from the freshwater plankton of Ceylon tends to emphasise the constancy of the occurrence of pseudovacuoles in the plankton species. The only case where individual variation occurs with respect to presence and absence of these structures is found in species (*M. æruginosa* Kuetz., etc.) which normally show them but where, in occasional colonies, some cells do and others do not possess them. However, the cells where these pseudovacuoles are absent show other signs of an abnormal, probably senescent or pathological condition (*e.g.* giving a coloration with corallin soda in distinction from the other cells which do not) and, moreover, traces of degenerating pseudovacuoles themselves are often revealed under the highest magnifications. Such cells are never found in a state of division. They are fairly constant in some forms of *Microcystis* and may perhaps be compared with the necridia of filamentous Cyanophycæ. Since they do not reproduce, it follows that the cells of new colonies will possess pseudovacuoles.

A definite cell-membrane, distinct from, and within the mucilage sheath, is generally stated to occur in the cells of the Chroococcaceæ (*e.g.* (11)). It corresponds in position with the "inner investment" of the sheath of filamentous forms, a layer which is insoluble in 33 per cent. chromic acid solution and which is said to consist of gelatinised form of protoplasm. Gomont(10) points out that unlike the mucilage of the surrounding sheath the inner investment has a constant composition among these higher Cyanophycæ. The various species of Chroococcaceæ vary appreciably with regard to the distinctness of the development of the cell-membrane. In *Glæocapsa alpina* (Naeg.) Brand(3) was unable to find it in normal cells, but observed quite freshly divided products of a division that were abnormally connected by a tubular bridge, and hence concluded that there was a definite cell-membrane in this species, although too fine to be visible in the ordinary state. Fritsch(8) found the membrane of *Glæocapsa* visible in older cells having a definite cell-sheath, but invisible in the young state. The same difficulty of demonstrating a membrane is

met with in many species of *Aphanocapsa* and *Microcystis* where the cells are embedded in copious mucilage. No doubt the optical properties of the latter are partly responsible for this. But it must also be remembered that the quality of the mucilage-vestment surrounding a cell would play a very considerable part in determining the surface tension and consequently the formation of a membrane. Where present the membrane is not separable from the protoplasm by plasmolysis, neither do stains differentiate it clearly. In all the species which we have examined, where there is any trace of a membrane at all, the new part of this layer does not grow into the protoplasm as a ring-like septum, finally closing and splitting into two as in the filamentous forms, but instead there is a constriction of the cell into two halves, the membrane growing round each daughter cell as it is completed. All such facts indicate that we are dealing with a hyaloplasm or modified outer layer of protoplasm rather than with a cell-wall.

THE MUCILAGE

The real cell-envelope in the Chroococcaceæ is the mucilage sheath. Mucilage is a common form of secretion in the Thallophyta and even in the higher plants, but its formation is generally localised and it helps to build up definite morphological features. The unicellular Cyanophyceæ, however, show very primitive features in their mucilage formation. Nearly all forms produce it at the periphery of the cell whilst in some, such as *Chroococcus turgidus* (Kuetz.) Naeg. it accumulates within the cytoplasm¹(5). Of course it is in the form of superficial deposits that it reaches its greatest development, and as such it soon takes on a protective function. In 1901 Lemaire(13) showed in species of *Chroococcus* and *Glæocapsa*, in some of the Nostocaceæ and in *Glæotrichia*, that the mucilage sheath showed great analogy with the pectic substances of higher plants. It readily took up basic anilin dyes, but had no affinity for acid dyes. In contrast it was found that in some Stigonemaceæ and less commonly in the Oscillatoriaceæ the sheath consisted mainly of a complex product which seemed to be due to the combination of an acid compound, filling the same rôle as pectic acid, with another substance of basic composition. The latter, which Lemaire calls schizophycose, is insoluble in zinc chloride and in dilute acids and alkalis. It is coloured by anilin blue and certain acid anilin dyes, but has no affinity for basic colours like ruthenium red, neutral red, etc. Finally, in some

¹ The so-called mucus vacuoles of many forms do not show reactions for pectins and so must be distinguished from the above.

Scytonemaceæ the sheath was found to contain cellulose, probably in a combined state, since it was insoluble in Schweitzer's solution. These cellulose-containing envelopes of Scytonemaceæ must not be confused with those of *Petalonema* and species of *Lyngbya* which also give a blue colour with zinc chloriodide and similar iodine reagents. For the reaction in these latter is due to a pigment, scytonemin, that can be dissolved out by means of eau de Javelle.

In 1910 a short account of the mucilage of one hundred and fifty Algæ was given by Virieux(17). Of the Cyanophyceæ examined *Chroococcus turgidus* (Kuetz.) Naeg. and certain species of *Phormidium* were found to have purely pectic sheaths. The results of Lemaire were verified with respect to the presence of schizophycose and cellulose in association with pectic substances in some of the filamentous forms, whilst certain membranes, *e.g.* those of *Schizothrix*, were found to be composed of pure cellulose. Virieux found callose in *Hydrocoleum heterotrichum*, whilst he also records schizophycose as occurring in *Glæocapsa*.

An examination of the mucilage of *Glæocapsa caldariorum* Rabenh. and of several unidentified species of the same genus, of *Microcystis æruginosa* Kuetz., *M. flos-aquæ* (Wittr.) Kirchn., and *Merismopedia elegans* A. Br. show them to consist almost entirely of pectic substances. They stain with basic anilin dyes and will absorb safranin, methylene blue and others from dilute solution. With formalin-fixed material safranin gives an orange, methylene blue a violet tinge. They are not stained by anilin blue, Congo red or eosin, nor do they react with zinc chloriodide and other similar iodine reagents. Methyl violet stains more or less strongly. They are dissolved in strong chromic acid.

It would seem that the secretion of mucilages of pectic substances is a primitive character. In the higher plants the first stage in membrane secretion is the formation of pectoses, giving the middle-lamella of tissue-cells. This, however, is soon superseded by the production of celluloses and then, generally at a somewhat later stage, of other substances. If we assume that in the lower plants such as the Cyanophyceæ, this ontogenetic series is represented by a phylogenetic one, then the Chroococcaceæ would take the most primitive place in such a series.

The question of mucilage secretion naturally leads over from a consideration of the properties of the individual cell to the characteristics of the colonies formed by the adhesion of these units into larger aggregates. Here, as would be expected from the above re-

marks on mucilage-secretion, we are dealing with phenomena of great variability. Colony form, which plays such a conspicuous part in the ordinary systems of classification, depends on several factors, viz. (1) the arrangement of the cells due to their special mode of cell-division, (2) the consistency of the mucilage, and (3) direct effect of the environment. The factors never act independently but sometimes one may predominate. Thus direct action of the environment may determine the spherical form of the colony in some species of *Microcystis* and *Cælosphærium* (cf. cell-shape), for it is significant that practically no large terrestrial colonies (e.g. those of *Aphanocapsa*) are spherical. It is instructive to compare the *Coccochloris* section of *Aphanothece* with the *Aphanothece* section of the same genus. The former is distinguished by a globose stratum, the latter has an amorphous one. The members of *Coccochloris* are mostly aquatic, whilst the majority belonging to the *Aphanothece* section are terrestrial.

Environment also acts indirectly by affecting the consistency of the mucilage, and indeed the actual process of secretion. The influence of stimuli on mucilage formation is well illustrated by comparing cultures of the same species in two different media. Thus Acton(1) records the development of asymmetrical envelopes in *Chroococcus turgidus* (Kuetz.) Naeg. when grown on damp soil: the unilateral growth was, in some cases, found to form a thick mucilage stalk longer than the cell itself. When grown in water, however, the mucilage investment is concentric in form. Similar cases have been observed in *Chroothece*. The extensive studies of Brunnthaler on *Glæothece rupestris* (Lyngb.) Born.(4) have shown that the amount of mucilage produced varies greatly in different media.

Consistency of mucilage may also vary with the nature of the species. This is generally assumed to be the case, and size of colony and distance apart of the cells may be given as diagnostic characters, at least in the case of those genera like *Cælosphærium*, *Microcystis* and *Merismopedia* where the colony has a somewhat definite shape. In nature, however, species of the two former may show single colonies in which the distance apart of the individual cells varies from several times the cell diameter to nearly nothing. *Merismopedia elegans* A. Br. may show a graded series in distance apart of the cells from one side of the colony to the other, although here the difference is not so pronounced. In *Microcystis æruginosa* Kuetz. and *M. ichthyoblabe* Kuetz. all transitions are observed from compact colonies to conditions where the mucilage is so diffuse that the cells are no longer held together.

Many species of Chroococcaceæ are characterised by the possession of envelopes which are not homogeneous. The heterogeneity is due generally to the differences in consistency and hence in optical properties of the successive layers of the envelope. Such stratified envelopes occur in nearly all the species or forms which have very dense and firm mucilage. In the simplest cases the alternating intensities of mucilage secretion producing such stratification are due to the alternating phases of vegetative and reproductive activity of the cell. Hence in certain colonial envelopes, such as those of *Glæocapsa* and *Glæothece* distinct layers of the envelope can be recognised as belonging to distinct cell-generations. But in the degree of development of such distinct sheaths there is very great variation, even in one and the same habitat. Brand(3), working on *Glæocapsa alpina* Naeg., found that this organism could pass over into a *status solutus* in which it was indistinguishable from an *Aphanocapsa*. Fritsch(8) observed that in *Merismopedia* where cell-division is very rapid the primary sheaths formed an envelope round several cells, whilst when division was slower the envelope only enclosed a pair of cells. We have noted similar variation in *Merismopedia*, sometimes correlated with the size of the colony and hence probably with the consistency of the mucilage (see Fig. 1 C, D). The colonies of the plankton species of *Microcystis*, too, are of interest in connection with the variability of the individual sheaths. In collections from the freshwater plankton of Ceylon, colonies of *Microcystis viridis* (A. Br.) Lemm. were sometimes found with very compact mucilage sheaths. In some of these the cells each had their own sheath, whilst in others they had not, and variation in this respect was found in one and the same colony. The more diffuent colonies showed no individual cell-sheaths.

Besides the lamellation of the membrane due to the existence of more or less individualised membranes for each cell-generation some envelopes appear, in optical section, to consist of numerous concentric layers. These may be formed by alternating periods of mucilage secretion of unequal intensity, but it is difficult to account for such periodicity. More likely they are due to the concentration into definite zones of some substance impregnating the mucilage. The phenomenon is probably almost identical with that of the formation of Liesegang's rings in gelatin. It is very variable in one and the same species. Thus in *Glæocapsa polydermatica*, a species which generally shows the layers in a very striking manner, we have observed all transitions from the stratified to the unstratified con-

dition (see also (4)). Where present, however, the character may be of systematic value as indicating the presence of a special substance in the mucilage comparable with the pigment of some sheaths, which, as we shall see, may disappear under certain conditions. Such a special substance is met with in the chitin of *Chroococcus macrococcus* Rabenh., which accumulates in special concentric zones of an otherwise pectic sheath (1).

THE PIGMENT

The phenomenon of mucilage-secretion is in some ways paralleled by that of pigment-secretion. It will be recalled that in the coloured plasma of all Cyanophyceae cells three coloured substances occur, namely, chlorophyll, carotin and phycocyan, the two former in the form of minute granules, the latter probably in solution. In those forms in which a definite central body occurs, the pigment is usually absent from this part, although Chodat (5) found in *Chroococcus turgidus* (Kuetz.) Naeg. that the central region was often rich in pigment. In addition to the ordinary pigmentation of the blue-green cell certain species of *Glæocapsa* and *Chroococcus* are distinguished by their different and more intense coloration. Kohl (12) has attempted to show that the pigmentation of these forms can be explained as the development of the three normal pigments in unusual proportions. Thus the violet and purple-red coloration of the cell in the subgenera *Rhodococcus* Hansg. and *Rhodocapsa* Hansg., the orange colour in *Chr. macrococcus* Rabenh. and the yellow in *Gl. paroliana* Bréb. may be explained as due to the preponderance of carotin with varying proportions of the other two pigments. No doubt this kind of variation is sufficient to explain the differences in cytoplasmic coloration. The latter may vary in one and the same species. Such is the case in *Gl. conglomerata* Kuetz. where the plasma varies between blue-green and brown, and in *Chr. pallidus*, *Chr. decolorans* and others where it varies from blue-green to yellow. The red pigment of *Glæocapsa alpina* Naeg. and of *Glæothece rupestris* (Lyngb.) Born. is found to disappear under certain circumstances (4) and we have noted a similar colourless state of the sheath as sometimes occurring in *Glæocapsa sanguinea* (Ag.) Kuetz. Colour variation is known in *Glæothece* to depend largely on ecological factors and in this respect there is no reason for supposing that the *Chroococcaceæ* differ essentially from *Gomphosphæria* (14) and *Oscillatoria* (18) where, however, the variability may be more striking.

But, whilst the variation in colour itself may be of little interest from a genetic point of view, the variation in its distribution may be

of much greater importance. The distribution of pigment with reference to the central region and peripheral protoplasm has already been referred to. The cases where, as in *Chroococcus turgidus* (Kuetz.) Naeg., the central portion of the protoplast may be as, or more, deeply tinged than the peripheral plasma represent a more primitive condition than those which have a central colourless region. For in the latter cases functional differentiation has become manifest between the layers of the protoplasm. If we assume that in allied Chroococcaceæ evolution may have progressed along similar lines, except that the central plasma has become coloured and the peripheral colourless, then it will be possible to connect genera like *Chroococcus* with others having a central chromatophore (e.g. *Chrootheca*). In view of the plasticity of the chromatophore region in the Chroococcaceæ there is no necessity to exclude organisms having a definite central chromatophore although some may equally well rank as reduced members of other Algal groups.

Other types of pigment distribution may be mentioned to illustrate the diversity of the group from this point of view. Whilst the majority of forms have their colouring matter diffused in the protoplasm or at least in very fine granules, some species of *Chroococcus* often show shining globules of pigment within the cell. In *Chr. macrococcus* (Kuetz.) Rabenh., for instance, there may be several such globules, in *Chr. rubrapunctus* Wolle one in the centre of the cell ("nucleus" of Wolle). In the former species the cells are often without these globules. The distribution of the pigment in these forms, however, seems to form an essentially distinct type and indicates a difference in the properties of the pigment or perhaps the protoplasm.

In other species of *Chroococcus* and in such species of *Glæocapsa* as *Gl. rupicola* Kuetz. and *Gl. purpurea* Kuetz. the red lipochrome is diffused throughout cell and sheath. This condition may be the most primitive one, comparable, in fact, with the cases where mucilage is secreted both within the protoplast and around it. But in most red species, and in all the brown species of *Glæocapsa*, the coloration is confined to the envelope. In some of these, at least, we are dealing with a pigment distinct from plasma-pigments. It has been given the name of glæocapsin and is said to be a chemical indicator like litmus, becoming rose-red, reddish-orange or bluish-red with hydrochloric acid, blue or blue-violet with caustic potash(19). In these Cyanophyceæ it is important to distinguish between envelope and plasma-pigments, just as in the higher plants a distinction must be made between plastid and cell-sap colours.

The variation of the pigmentation of the *Chroococcaceæ*, together with the saprophytic tendencies of the group, would lead one to expect colourless representatives to be not uncommon. These are probably found in such bacterial genera as *Pediococcus* and *Sarcina*. It is of interest to note the extensive lipochrome production in certain species of the latter.

PLANES OF CELL-DIVISION

Amongst the characters first adopted by the older algologists in distinguishing the various forms of unicellular blue-green Algæ the spatial relation of the successive planes of division to one another played the most important part. This was perhaps due to the very obvious differences which may arise in colonial forms, as a result of differences in relation of the division planes, so that the distinction, for instance, between a *Merismopedia* and an *Aphanocapsa* is in this respect a very striking one. It is noteworthy that the various systems of classification of the bacteria have also been based largely on the character of the division planes, but these systems have met with adverse criticism since certain filamentous forms show several possible types at different stages. The same objection might be raised in the case of the *Chroococcaceæ*. For at first sight it might seem that examples are not wanting in which cell-division in definite planes at right angles may give place to division in almost any plane. The fact that *Glæocapsa* can pass over into an *Aphanocapsa* condition has already been mentioned. In all such cases, however, a critical examination of the actual phenomenon does not indicate that there has been any change in the actual relation of the division planes to one another (*i.e.* in the polarity of the cell). *Aphanocapsa*-forms are all characterised by the diffuent character of the mucilage. Within such mucilage rotation of the cells would become much easier than in the more compact types (*Glæocapsa*, *Merismopedia*). It is not easy to prove that such rotation occurs in *Aphanocapsa* because the cells are isodiametric, but in the similar diffuent *Aphanothece*-types with their elongated cells it is quite easy to observe. In Fig. 1 the shifting of two cells with reference to one another is shown diagrammatically.

In all but one of the families of the filamentous Cyanophyceæ there is absolute constancy of cell-division in the plane at right angles to the axis of the filament. Even when branching takes place this plane is not departed from, the well-known "false-branches" thus being formed. In the branching of the *Stigonemaceæ*, however, we get division planes at right angles to the normal direction. It is as

if the cells were capable of dividing in more than one plane but in the ordinary filament were inhibited from doing so. It is not possible to offer a complete explanation of this inhibition, nor is this the place to deal with these interesting filamentous Cyanophyceæ. But it may be noted that where cells are so closely approximated as in these filamentous forms, by reason of their association in one dense mucilage sheath, that interaction of the cells on one another is to be expected. Most writers are agreed that passage of substances in solution may occur in the filamentous forms (18).

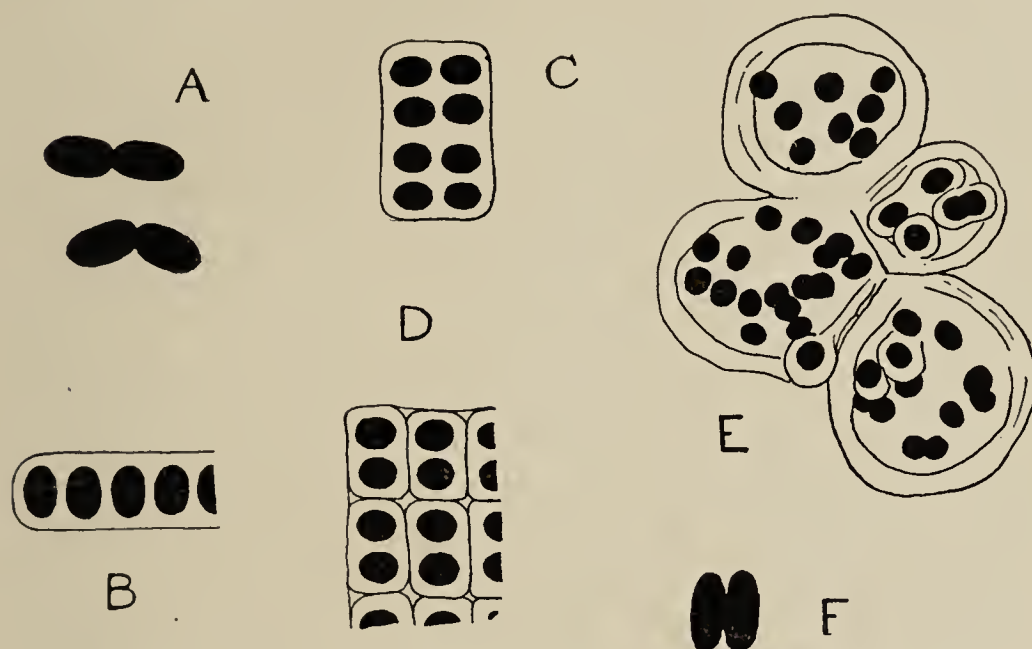


Fig. 1. Diagrams, mostly constructed from Camera-lucida drawings.
Protoplasm black.

- A. *Aphanothece* sp. Final stages in the division of a cell to show elongated character of daughter cells and shifting of their axes on completion of division.
- B. *Merismopedia elegans* A. Br. View of edge of colony to show elongation of cells.
- C. *Merismopedia* sp. Young colony without individual membranes.
- D. *Merismopedia* sp. Old colony to show individual membranes.
- E. *Microcystis viridis* Lemm. Colony to show variation with regard to presence and absence of individual membranes and in distance apart of cells.
- F. *Holopedium irregulare* Lagerh. (after Kirchner). Two cells from side.

SUMMARY AND CONCLUSIONS

From the foregoing it would appear possible to draw certain conclusions bearing on the classification and evolution of the group of the Chroococcaceæ. Many of the features studied here have played an important part in the taxonomic treatment of the family. It would appear that certain characters, such as the orientation of the planes of division, the form of the cell, the size of the cell, can be,

to a large extent, relied upon as systematic distinctions, as was recognised by the earlier workers. The presence of pigments, pseudovacuoles and certain types of stratified membranes may be also of importance, when the life-history is fairly well known. It also appears that cytological characters such as the degree of differentiation of the protoplasm and particularly the distribution of the pigment are very significant, although such characters have hitherto received little recognition in schemes of classification. On the other hand, the character of the stratum or colony has only slight morphological significance, although this has been largely relied upon by systematists. The distinctions between many species and even genera must be regarded as differences in life-history. So although some specific and generic forms are convertible one into the other, yet for any given organism, growing under normal conditions, there may be one predominant morphological form. Another organism may be capable of presenting precisely the same phases of life-history but the predominant phase may be different from that of the first organism. In general, among the Chroococcaceæ we are dealing with quantitative rather than qualitative differences. The number of genetic factors producing qualitatively different effects in the organisms investigated need be relatively small. It is not possible, of course, to enumerate these factors, but it can be seen from the foregoing analyses of the morphology that they are such as affect rate of growth, secretion of various substances, and those regulating the surface of the cell or affecting the polarity of the protoplasm. These characters, especially the first two, are also likely to be influenced by external factors. But that there are internal factors playing an important part in determining the structure of the organism is obvious from the fact that two or more forms, growing under the same conditions, sometimes even in the same stratum, may remain quite distinct, even after growing side by side for many months.

The group of the Chroococcaceæ shows such a number of features that may be regarded as primitive that it is unlikely that as a whole the family consists of reduced forms. It seems fairly clear that the family is practically homogeneous from the phylogenetic point of view. The differences rather than the resemblances of the various forms have been dealt with in the previous pages, but in most cases true transitions have been found between the extreme types. Among the most prominent indications of the primitive character of the group are the production of several pigments in the plasma, the variability of these pigments in amount and distribution, the un-

specialised character of the envelope, the pectic nature of the latter, the variability in the differentiation of the layers of the protoplasm, including the plasma-membrane, so that sometimes the cell may be homogeneous. The lack of a definite chloroplast and a nucleus is also of importance in connection with the primitive nature of the group. Incidentally, it seems altogether gratuitous to compare the central region with a nucleus, as suggested by many writers, especially as the existence of chromosomes and even of chromatin is more than doubtful. It might better be compared with the endoplasm of many Flagellates and Rhizopods. In connection with the lack of nuclear structure it must be pointed out that this by no means precludes a precise mechanism of heredity; even in the higher plants non-nuclear inheritance is held to be possible (*e.g.* plastid inheritance).

The complete absence of flagella in the Cyanophyceæ is a remarkable feature, which may or may not be a secondary character. Whilst it is true that the majority of Algæ and Protozoa have been shown by recent work to be connected by all transitions with Flagellate forms, yet most of these organisms show a flagellate stage at some period in their life-history. In many ways the Chroococcaceæ are as primitive or more so than the majority of Flagellata, although their structure is of quite a different type. In their mode of reproduction by binary fission rather than by multiple division the Chroococcaceæ are less primitive than the allied Chamæsiphonaceæ, although the latter are more advanced with respect to the thallus, in which apical and basal portions are differentiated.

The general principle, that multiplicity of similar parts or structures is more primitive than the condition in which such similar parts are few, may be applied to the axes of cell-division. The lowest members of the Chroococcaceæ, then, are those showing division in three planes at right angles to one another (*Chroococcus*, *Glæocapsa*). Reduction of the axes to two (*Merismopedia*, *Holopedium*) or one (*Synechococcus*) may take place. Following the polarisation of growth especially developed in connection with the restriction of the division axes, the cell may become modified in form (*Aphanothece*, *Tetrapedia*). Further modification may take place by early constriction of the cell in the planes of division (*Tetrapedia*), this being paralleled in the cell-form of the majority of Desmids. Filamentous forms are made possible by the restriction of the planes of division; the method of origin of the Oscillatorian filament has been indicated. Amorphous types of colony may arise in the Chroococcaceæ from definite types by the mucilage becoming diffuent (*Aphanocapsa*, *Aphanothece*).

Denser types of mucilage may show individual cell-sheaths. The culmination of this type in *Glæocapsa* and *Glæothece*, where each cell-generation shows its individual sheath, may be regarded as secondary, since so many of these forms are only semi-aquatic or even completely terrestrial. In general, the larger colonial forms tend to develop small cells (see Table II). When not united to form definite colonies, however, the plant-body may sometimes attain a moderate size in the form of separate cells (*Chr. macrococcus* (Kuetz.) Rabenh.).

In agreement with the general principle that homogeneity, either physiological or morphological, gives rise to heterogeneity in the course of evolution, we can trace various phylogenetic stages in the differentiation of the protoplast and in the distribution of pigment. The latter may accumulate in both protoplasm and envelope or may be confined to either. Where only plasma-pigmentation occurs the colouring matter may be equally diffused or concentrated either in the peripheral or the central plasma. The type having a peripheral chromatophore has survived in all the higher Cyanophyceæ. The type with central coloured plasma may have led to those forms which have a definite central star-shaped chromatophore.

In conclusion the writer wishes to thank Professor F. E. Fritsch both for the loan of material and for several helpful suggestions and Professor R. C. McLean who has also taken considerable interest in the above work.

TABLE I

Variation in cell-diameter in collections from single habitats

The headings indicate the dimensions of the classes, measured in microns. Under the dimensions of each class is given the number of individuals in the class. For each species 100 individuals were measured.

Species	1	2	3	4	5	6	7	8	9	10
<i>Glæocapsa crepidinum</i> (Rabenh.) Thuret	—	17	50	31	2	—	—	—	—	—
<i>Gl. Ralfsii</i> (Harv.) Lemm.	—	3	28	55	13	1	—	—	—	—
<i>Gl. polydermatica</i> Kuetz. (large form)	—	—	—	—	11	20	28	25	11	5
<i>Glæocapsa</i> in thallus of lichen <i>Psorotichia sanguinea</i>	—	—	—	55	35	10	—	—	—	—
<i>Glæocapsa</i> in thallus of lichen <i>Jenmania Gæbeli</i>	—	4	23	54	16	3	—	—	—	—
<i>Chroococcus cohærens</i> (Bréb.) Naeg.	—	—	2	12	26	32	16	6	6	—
<i>Merismopedia elegans</i> A. Br.	—	—	—	7	54	35	4	—	—	—
<i>Cælosphærium</i> sp.	—	—	—	—	1	16	56	22	3	2
<i>Microcystis æruginosa</i> Kuetz.	—	—	48	50	2	—	—	—	—	—
<i>M. pallida</i> (Farlow) Lemm.	—	—	—	2	29	45	18	6	—	—

TABLE II

Dimension recorded (7, 14)

In the case of elongated cells only the width is given.

Chroococcus:

<i>Chr. turgidus</i> (Kuetz.) Naeg.	13-25 (rarely 40)
(incl. <i>Chr. giganteus</i> W. West, to 60)	
<i>Chr. minutus</i> (Kuetz.) Naeg.	5-7
<i>Chr. varius</i> A. Br.	4-8
<i>Chr. insignis</i> Schmidle	10-12
<i>Chr. macrococcus</i> (Kuetz.) Rabenh.	25-50
<i>Chr. caldarium</i> Hansg.	3-6
<i>Chr. limneticus</i> Lemm. agg.	8-13
<i>Chr. parallelepipedon</i> Schmidle	3

Glæocapsa:

<i>Gl. montana</i> Kuetz.	2-5
<i>Gl. salina</i> Hansg.	3-6
<i>Gl. caldarium</i> Rabenh.	3-8
<i>Gl. æruginosa</i> (Carm.) Kuetz.	2-3
<i>Gl. rupestris</i> Kuetz.	4-9
<i>Gl. crepidinum</i> (Rabenh.) Thuret	4-7
<i>Gl. Ralfsii</i> (Harv.) Lemm.	4-7
<i>Gl. magna</i> (Bréb.) Kuetz.	4.5-7
<i>Gl. rupicola</i>	4-6
<i>Gl. purpurea</i> Kuetz.	1.5-2.5
<i>Gl. alpina</i> (Naeg.) Brand emend.	2.5-8

Microcystis:

<i>M. elabens</i> (Meneg.) Kuetz.	1-1.5 (rarely 3-4.5)
<i>M. marginata</i> (Meneg.) Kuetz.	3-4
<i>M. firma</i> (Bréb. et Lenorm.) Rabenh.	0.7-2.3
<i>M. flos-aquæ</i> (Wittr.) Kirchner	4-6.5
<i>M. æruginosa</i> Kuetz. agg.	3-6
<i>M. scripta</i> (Richter) Lemm.	5-7
<i>M. ochracea</i> (Brand) Lemm.	4-6.5 (rarely 8)
<i>M. fuscolutea</i> (Hansg.) Migula	3-4
<i>M. holsatica</i> Lemm. agg.	0.3-1
<i>M. stagnalis</i> Lemm.	1-2

Merismopedia:

<i>M. elegans</i> A. Br.	5-6
<i>M. Marsonii</i> Lemm.	1.3-2

LITERATURE CITED

- (1) ACTON, E. Observations on the Cytology of the Chroococcaceæ. *Ann. Bot.* 28. 1914.
- (2) BESSEY, C. E. A Synopsis of Plant Phyla. *Univ. Studies, Nebraska*, 7. 1907.
- (3) BRAND, F. Der Formenkreis von *Glæocapsa alpina* Naeg. *Bot. Centralbl.* 83. 1900.
- (4) BRUNNTHALER, J. Der Einfluss auszerer Faktoren auf *Glæothece rupestris* (Lyngb.) Born. *Sitzungsber. der kaiserl. Akad. in Wien, Mathem.-naturw. Klasse*, 119. 1909.
- (5) CHODAT, R. *Chroococcus turgidus*. *La Nuova Notarisia*. 1896.
- (6) FISCHER, A. Die Zelle der Cyanophyceen. *Botan. Zeitung*, 63. 1905.
- (7) FORTI, A. Myxophyceæ. Vol. 5 of De Toni's *Sylloge Algarum*. 1907.
- (8) FRITSCH, F. E. Studies on Cyanophyceæ. II. *Beihefte z. Bot. Centralbl.* 18. 1905.

- (9) GARDNER, N. L. Cytological Studies in Cyanophyceæ. *Univ. of California Publications, Bot.* **11**. 1906.
- (10) GOMONT, M. Recherches sur les enveloppes cellulaires des Nostocacées filamenteuses. *Bull. Soc. bot. de France*, Sér. 2, T. **10**. 1888.
- (11) KIRCHNER, O. Schizophyceæ in Engler u. Prantl, *Die natürl. Pflanzenfam* Teil I, Abt. 1 a. 1898.
- (12) KOHL, F. G. *Über die Organisation und Physiologie der Cyanophyceenzelle*, u. s. w. Jena, 1903.
- (13) LEMAIRE, A. Recherches microchimiques sur la gaine de quelques schizophycées. *Journ. de Bot.* **15**. 1905.
- (14) LEMMERMANN, E. *Algen in Kryptogamenflora der Mark Brandenburg*, **3**. 1907.
- (15) OLIVE, E. W. Mitotic division of the nuclei of the Cyanophyceæ. *Beihefte Bot. Centralbl.* **18**. 1905.
- (16) TILDEN, J. Minnesota Algæ. Vol. *Myxophyceæ Minnesota*. 1910.
- (17) VIRIEUX, J. Sur les gaines et les mucilages des Algues d'eau douce. *Comptes rendus Acad. des Sc.* **151**. 1910.
- (18) WEST, G. S. *Algæ*. Vol. 1. Camb. Bot. Handbooks. Cambridge, 1916.
- (19) ZIMMERMANN, A. *Botanical Microtechnique*, trans. J. E. Humphrey. New York, 1893.

REVIEW

Lichens. By ANNIE LORRAIN SMITH, F.L.S. Pp. xxviii and 464, with 135 figures in the text. (*Cambridge Botanical Handbooks*.) University Press, 1921. Price 55s.

The publication of this second volume of the *Cambridge Botanical Handbooks* has been delayed owing to war conditions, and if this delay is, in any way, responsible for its excellence, all students of lichenology must congratulate themselves that it has occurred. It is so full of matter that one marvels at the wonderful application of the author in collecting and arranging the work on the various aspects of lichenology into critical articles, and then weaving these articles together so as to form a connected whole, which may be read with pleasure and profit, not only by a lichenologist, but also by a general botanical reader.

The Bibliography of works consulted in the preparation of the volume is an extensive one, and includes all the important books and papers on lichens. Very few British articles on lichenology, whether important or otherwise, are omitted. One cannot remember a dozen which are not included, and such omissions as there are involve no great sacrifice to the completeness of the work. Even Wheldon's article on mites using the pits of immersed apothecia for their homes is included, though such a paper, interesting as it is, seems rather out of place in a general work on lichens.

The terminology is much simplified as compared with that employed by Leighton and Crombie, and the author must be congratulated on the ability with which she has lucidly explained the morphology of lichens without requiring an extensive glossary. A glossary is given, but many of the terms in it are sparingly, if at all, used by the author. The value of Crombie's monograph was largely discounted by the technical jargon he used.

The book is divided into ten chapters, dealing with the history of lichenology, the algal and hyphal constituents of the thallus, the morphology of various types of thallus and associated structures, the methods of reproduction, the physiological aspects (including an account of the chemical substances formed by the cells), the bionomics of duration, dispersion and parasitism, the probable phylogeny of the various groups, the classification and distribution, the ecology, and finally the economic and technical uses. In the last chapter many interesting, though obsolete, uses are quoted. The only use (except for litmus) to which one has seen lichens put in the south-west of England is wreath-making, and this is not mentioned. Usneas are the chief lichens used, but Ramalinas and *Evernia prunastri* are also employed. *Cladonia rangiferina* is referred to on p. 401 as the "favourite food" of the reindeer. One may be pardoned for suspecting that it is their favourite food, only because it is Hobson's choice. Zopf's statement (quoted on p. 400) that reindeer absolutely refuse to eat *Cladonia sylvatica* is rather misleading. If they are able to distinguish between *C. rangiferina* and *C. sylvatica*, they are cleverer than most lichenologists.

The table of contents is a special feature and occupies twelve pages. It gives full information on the sections treated in each chapter, thus acting as a guide to the student seeking information on a special point, and leaving the index free for a list of the authors and plants referred to in the work.

The author often gives the views of other lichenologists and states the evidence on which these views are founded, without committing herself. When she expresses her personal views, they appear to be sane and safe ones, which harmonise with a broad and general knowledge of the subject. Much of the work has been done by specialists; sometimes by a field-worker with little experience of experimental work, at others by a laboratory worker who has concerned himself little with the natural conditions under which the plants grow, so that his work with artificial cultures may have only a specious value. The author's knowledge of the various parts of lichenology, in addition to her studies of micro-fungi, has enabled her to coordinate and correlate the work of such specialists in an efficient manner.

Much of the work on nutrition discussed on pp. 39-43 is incomplete or unconvincing. Most of the nutritive experiments were carried out with *Xanthoria parietina*, or some other lichen, which prefers situations where organic material is abundant. The results of some of the experiments merely prove that the separated symbionts prefer a good diet to what is a starvation one for them, but one on which many lichens are able to exist. The occurrence of lichens in alpine situations, on more or less vertical rock-faces, can scarcely be analysed by artificial methods of growing the separated gonidia or hyphæ in comparatively rich nutrient solutions. Lichen hyphæ are stated on p. 47 to be usually thicker-walled than those of a true fungus. If this were a constant "spot" distinction the difficulty of distinguishing between a lichenoid fungus and a fungoid lichen would be simplified. The view that the fungus is parasitic on the alga, which has been recently revived by Elenkin, receives no support. Any lichenologist knows that penetration of the algal cells by the fungus is an unusual occurrence, and, though empty algal cells are often frequent, they are much less frequent than in a scraping of *Protococcus* taken from a tree-trunk.

The alterations of the algæ, particularly of *Scytonema* and *Trentepohlia*, when they become symbiotic partners with fungal hyphæ, is well expressed on p. 62. In the case of *Placynthium nigrum* the alga seldom shows its proper character; sometimes it appears like a *Tolypothrix*, but more often it is still less like *Scytonema*. The algal cells in

Polychidium muscicolum and *Leptogium microscopicum* seem very similar, though those of the former are given as *Scytonema*, and those of the latter as *Nostoc*. Figures of the algal symbionts are given from the free algæ. It would have been better if illustrations of the actual gonidia had also been given, since a young lichenologist may be led to expect to find algal symbionts in the lichen like those figured.

Many instances of what various workers have called saprophytism and parasitism are mentioned. Some of the so-called saprophytes amongst lichens are no more entitled to the name than are radishes from a well-manured garden plot, and some of the examples of parasitism are scarcely more so than when plantain smothers other plants and feeds on the derived humus; others, in which digestive action precedes the actual covering of the host, are better examples. Malme's view (quoted on p. 261) that "*Buellia verruculosa* and *B. æthalea* may be living on the thallus of *Rhizocarpon distinctum* with which they are constantly associated," is not supported by the distribution of these lichens in the British Isles. When Miss Smith expresses her own views on these so-called parasitic and saprophytic lichens, they are generally of a sceptical or qualifying nature, though it is not always easy to perceive how far she agrees with the work and views she gives an account of.

The colour of lichens has an excellent section devoted to it, and the effect of light on this colour is referred to in another section of the chapter dealing with physiology. The darkening or deepening of the colour is attributed to the influence of light. In some lichens it may not be the only factor, the lack of water and nutriment may also contribute to the darkening. The dark colour of such plants as *Rhizocarpon confervoides*, *Buellia impressula*, and *Lecidea latypea* on shingle, is due to the predominance of the hypothallus, the nutrition being deficient for the proper formation of the thallus. The change in the colour of the apothecia (p. 245) is of frequent occurrence, and sometimes is well marked where a number of stones have lain heaped together for many years. The apothecia on the illuminated portion may be very dark whilst those which are shaded are pale in colour. The darkening of old apothecia, e.g. *Candelariella vitellina*, may be due to decomposition products.

Those who uphold Church in his views of a direct evolution of lichens from algæ, may derive some consolation from the following passages, when isolated from their contexts. "In *Verrucaria mucosa*...and in some other sea-washed species the arrangement of the tissue elements recalls that of crustaceous Florideæ such as *Hildenbrandtia*, *Cruoria*, etc." (p. 73). "Mechanical tissue scarcely appears among fungi" (p. 105). "These attaching sheaths (of *Usnea*, etc.)...may be more truly compared with the primary thallus of the red algæ, *Dumontia* and *Phyllophora*, which are similarly affixed to the substratum" (p. 111). "Such a view might be possible if we could regard lichens and Florideæ as derived from some common ancestor" (p. 179). "The hyphæ travel in the sheath alongside the cell-rows and the symbiotic plant retains the tufted form of the alga as in *Lichina* with *Rivularia*, *Leptogidium* with *Scytonema*, and *Ephebe* with *Stigonema*" (p. 284). In the phylogenetic chapter, and more definitely in an appendix, Miss Smith opposes these views, and in so doing voices the opinions of lichenologists in general. Even those who have seen "the shaggy covering of Lichen on the cliffs facing the Atlantic" ("The Lichen as Transmigrant," *J. of Bot.* 1921, p. 45) find little evidence in support of Church's views and much in conflict with them.

A large amount of space is given to Zahlbruckner's method of classification. The appreciation given to it is well deserved, as it is the most natural and thorough grouping of the lichens which has been attempted. Miss Smith's lucid description brings it within the range of every British student. Some desirable information has been omitted, probably owing

to considerations of space. One would have liked to know what British species are referable to the genera *Polyblastopsis*, *Blastodesmia*, *Phæographina* and *Pilocarpon*, the position of *Bilimbia*, why *Acrocordia* is merged in *Arthopyrenia*; in fact, a more detailed account of Miss Smith's personal views would have been very acceptable. Her caution in regard to some parts of Zahlbruckner's classification can easily be understood; still, the grouping of the lichens with many-spored asci in *Acarosporaceæ* seems justifiable, and the separation of *Xanthoria* from the *Physcia* group is supported by the differences in the septation and colour of the spores, of which the importance in classification has scarcely been sufficiently appreciated by our older British lichenologists except Mudd. With regard to Zahlbruckner's lumping together of a number of genera into *Lecideaceæ* and *Lecanoraceæ* one may be heterodox and less in agreement. Too much importance may be assigned to the systematic value of "the distinction between lecideine and lecanorine apothecia," insisted upon on p. 183. *Lecanoraceæ* may be polyphyletic from lecideoid ancestors and an extension of the principle which includes *Blastenia* with *Placodium* and *Buellia* with *Rinodina* seems justifiable. *Candelaria* and *Candelariella*, despite the absence of parietin, appear to have more affinity with *Xanthoria* and *Placodium* than with the groups under which they are placed.

In a few instances one is not prepared to accept Miss Smith's conclusions, as for example, in the general summing-up of the evidence given in the case of pycnidia *versus* spermatogonia, where the former is favoured. The evidence, however, on both sides is given impartially and the reader may use his own judgment. The evidence of Stahl, Darbishire, and others, as to contact between the spermatium and trichogyne, is not challenged. The author's chief point against the spermatium being a gamete rests on its power of germination. The possibility of a gamete settling down to produce a new plant is familiar to algologists, and there does not appear to be any inherent improbability in the reappearance of such an ancestral character, when the spermatium is cultivated. The differences between the colourless, polarilocular spores of *Xanthoria*, and the brown, one-septate spores of *Physcia*, appear greater than is suggested on p. 188. According to the text, the median septum arises as an ingrowth from the sides of the cell. In *Xanthoria* the ingrowths do not completely meet, so that a canal is left between the two polar chambers. In *Physcia* the ingrowths meet and two distinct chambers are formed. The illustrations (Fig. 107) show a median septum crossing not only the central canal, but also traversing the ingrowth from the sides of the cells, so that the median septum of *Physcia* traverses what is considered to correspond to a median septum in *Xanthoria*. The *Xanthoria* family with *Teloschistes*, *Xanthoria*, *Placodium*, *Callopusia* and *Blastenia*, seems to be a distinct family from that of *Physcia* with the more or less corresponding genera of *Anaptychia*, *Physcia*, *Dimelæna*, *Rinodina* and *Buellia*. The references to similar cell-division in *Algæ* would have been better if *Sphæroplea* had been mentioned instead of *Cladophora*; in the latter the wall is completed, whereas in *Sphæroplea*, an open central pore is sometimes left. In some *Siphonales* (e.g. *Callipsygma*) an open pore is left; in *Codium* the stopper is incomplete when young and complete at an older stage.

There are few inaccuracies or inconsistencies in the text, and for some of these the author is not responsible, as she is quoting the views or work of others. Some accident causes *Collema nigrescens* to have a distinct cellular cortex on p. 161. According to p. 371 "lichens alone are able to live on bare rock," whilst it is stated on p. 394 that "in tropical countries the first vegetation to settle on bare rocks would seem to be blue-green gelatinous algæ." On p. 374 *Collemopsis* is said to be

"the only Xanthocapsa associate that is silicicolous" and on the opposite page *Psorotichia lugubris* is listed. The statement on p. 250 that rust lichens "grow on rocks that are frequently under water" needs qualifying, as only one of the four examples given grows in such situations, and other exceptions could be mentioned. The formula of such a highly complex substance as triethylprotocetraric acid may still be given as $C_{20}H_{18}O_9$, even though it is an empirical one (p. 221). The "yellow-coloured medulla of *Parmelia subaurifera*" (p. 226) is usually white in southern plants. In the *résumé* of the work done on the shingle lichens at Blakeney Point, *Lecanora badia* is given as abundant. During some observations on the shingle beds in 1921, *L. badia* was not seen, though *Lecania prosechoides* was abundant and is probably the lichen meant. *Placodium lobulatum* was also abundant on the low shingle and is probably the lichen referred to as *Lecanora citrina* var. *incrustans*.

Some biologists may reasonably object to the use of copulation for contact or fertilisation (p. 160, etc.), rhizoid for rhizina (pp. 92 and 93) and prothallus for hypothallus or protothallus (p. 187), as their use may cause confusion, or are scarcely in accordance with their use in other branches of biology. The physiological meaning of the statement quoted on p. 224, about waste products and products of deassimilation is rather obscure. Bitter's suggestion of hybridity between *Parmelia physodes* and *P. tribulosa*, owing to the intermingling of their soredia (p. 146), would be more convincing if the specific segregation of these two plants was established. The phylogeny of the Coniocarpineæ (p. 276) may be more easily explained by assuming that their fungal ancestors belong to an extinct group.

The general get-up of the book is excellent; headings, spacing, wide margins and illustrations give an artistic setting which such a great work deserves, but (and there is a very objectionable but) the price is much too high. The work ought to be readily accessible to every student of plant-life, and the high price will prevent such a desirable consummation. Perhaps it would have been advisable to place some of the more critical matter in smaller type, if such a course would have resulted in a cheaper book. Much of the matter dealing with the chemical contents of the cell could have been so treated.

A general consideration of the book makes one realise what a great diversity of opinion exists on very many lichenological questions. Even in regard to the distribution of lichens in the Polar regions, Darbishire and Hue are at variance. According to the former 50 per cent. of Antarctic lichens are also Arctic, whilst Hue states that 81 per cent. of Antarctic lichens are special to this southern region. The magnitude of the task in considering and coordinating such a variety of views so as to form a connected story may well have appalled a stout heart, and we may be proud of the fact that it has been so splendidly accomplished by one of our own botanists.

W. WATSON.

A METHOD FOR INDUCING PROTOPLASMIC STREAMING¹

By WILLIAM SEIFRIZ

THE teacher of biology is often confronted with the task of finding material with which to demonstrate protoplasmic streaming. The material usually employed for this purpose is *Elodea*. Frequently, however, one may search for hours without success, or, if streaming cells are located beforehand, they are likely as not quiescent at the very moment that they are needed for demonstration. It should, therefore, prove of advantage to know some means by which streaming can be induced, so that one can be certain of having the desired material ready when it is needed.

While studying the reaction of protoplasm to various reagents, I observed an extraordinary amount of streaming, often at a very high rate, in the cells of *Elodea* which had been treated in certain of these reagents. Once having ascertained the comparative resistance of the cells to a reagent, it was an easy matter to obtain an abundance of streaming by treating the cells for a definite length of time.

In addition to a great amount of active streaming, the type of streaming was frequently abnormal. Indeed, no less than half a dozen distinct types of streaming were observed, and these were often in the majority as compared with the normal rotational type usually seen in the cells of an *Elodea* leaf.

Methyl alcohol was the chemical in which a pronounced stimulation of protoplasmic streaming was first observed.

The length of treatment in solutions of alcohol varies not only with the per cent. of solution used but also with the resistance of the cells to alcohol. Those cells situated in the centre of a leaf are least resistant. The two or three hundred cells situated in the very base of the leaf are most resistant. Not only do the cells in one leaf vary greatly in their sensitivity to alcohol, but different leaves on the same shoot and different shoots collected at different times and in different localities show a greater or less resistance to the toxic effect of alcohol.

¹ The research work from which the data here presented were obtained was done at the Botanical Institute of the University of Geneva where the writer enjoyed the privileges of a guest through the courtesy of Professor R. Chodat.

A 10 per cent. solution of alcohol will kill nearly all of the cells of an Elodea leaf within two hours (80 per cent. are killed within one hour). The time is too brief in which to arouse the cells to activity before death ensues. In 8 per cent. alcohol several hours' treatment is usually enough to arouse the cells to activity. Eighteen to twenty-four hours is sufficient in a 5 per cent. solution, while from two to three days is necessary in 3 per cent. alcohol. The lower grades, viz. 3 per cent. to 5 per cent., with long treatment are more satisfactory. In a leaf which has undergone such treatment about one-half of the cells will exhibit active streaming. In some instances I have observed nearly every cell in the leaf, with the exception of the few which have been killed, in an active state of streaming. In many of them the chloroplasts are racing around in the cell at a remarkably high speed.

While the amount and rate of streaming is very pronounced in alcohol treated cells, the most interesting feature of this stimulation to protoplasmic activity is the great variety of abnormal types of streaming. The usual type of streaming in a normal cell, as seen in optical section, is a movement of cell inclusions around the inner surface of the cell wall in a plane parallel to the surface of the leaf, and ordinarily all chloroplasts take part. In an alcohol treated cell, however, the chloroplasts may be grouped in a variety of ways.

One very common type of streaming in these alcohol treated cells is that in which the chloroplasts are arranged in the form of a belt encircling the protoplast at its centre in a plane perpendicular to the surface of the leaf. When the belt is broad the protoplast resembles a revolving cylinder. At times the belt consists of two apparently independent streams moving at different rates. Again, there may be two such belts of chloroplasts, one at each end of the cell situated on the very tip of the plasmolysed protoplast, or intermediate to the ends in a plane oblique to the cell axis. What is especially interesting is that two such belts in a single cell revolve, in some instances, in *opposite* directions.

The second reagent in which I observed a stimulation of protoplasmic streaming in Elodea cells was saponin, including the similar glucosides, smilacin and senegin. The results are very like those just described for alcohol; namely, a great amount of streaming, much of it at a remarkably high rate, and many abnormal types. The saponins used in my experimentation were all chemical preparations; but for obtaining a saponin suitable for treating Elodea leaves in order to arouse the cells to activity it is convenient to extract the saponin

from Panama wood (*Quillaria saponaria*) which is usually to be had at a chemist's. Five grams of the wood may be boiled in 100 c.c. of water for fifteen minutes. (Spring water is preferable to distilled water, unless the latter is absolutely pure. Water from a copper still will kill the majority of the cells in an *Elodea* leaf over-night.) The extract obtained from Panama wood will, in 100 c.c. of water, be of the desired strength for treating the cells for one or two days. If chemical preparations of these glucosides are used the most satisfactory per cent. of solution is 2 where brief treatment is desirable. In the case of senegin a weaker solution (1 per cent.) is preferable, owing to the greater toxicity of senegin.

The time of treatment again, as in alcohol, varies with the physiological condition of the leaves. Eighteen to twenty-four hours is usually sufficient in a 2 per cent. solution of saponin, or 1 per cent. of senegin. Abundant and very active streaming of a great variety of types results from such treatment.

The third chemical which proved to be so strong an activator to protoplasmic streaming was strontium chloride—also barium chloride which is very similar to strontium chloride in its effect on such physical properties of protoplasm as viscosity, permeability, osmotic pressure, and streaming.

Both strontium and barium are highly toxic to protoplasm; but barium more so than strontium, therefore, a lower concentration of barium than that which is isosmotic with the maximum per cent. of usable strontium chloride should be employed. The usable concentration of any salt for long treatment must, of course, be slightly below the critical concentration of that salt, *i.e.* below the concentration which will just cause plasmolysis. For strontium chloride this is about 2 per cent., for barium 2.7 per cent. Owing to the greater toxicity of barium a 1 per cent. solution is more satisfactory for inducing streaming.

The length of treatment varies with the sensitivity of the leaves, from one to three days usually being necessary. Eighteen hours is sometimes sufficient to arouse the cells to moderate activity in strontium, and often to pronounced activity in barium.

All the types of streaming so far described are to be seen in strontium chloride and barium chloride treated cells. There may be a stream of chloroplasts moving around a group of quiescent chloroplasts, the latter showing no tendency to be drawn into the stream even when the moving chloroplasts rub against them; yet later these same quiescent chloroplasts may of themselves commence streaming

and establish a second and independent rotating circle within the former outer one.

Another interesting type is that in which the stream of chloroplasts proceeds part way up the side of a cell and then turns, crossing the cell diagonally back to the corner opposite that from which it started, then across the end, thus leaving about one-half of the cell, in which chloroplasts are also present, in an inactive state. One of the most interesting features of a strontium treated cell is this very tendency of the salt apparently to affect one region of the cell more than another.

The chloroplasts may also be arranged in the form of a large disk which slowly turns.

One unique arrangement observed was that in which there was a broad belt of chloroplasts revolving in a plane perpendicular to the surface of the leaf, and another group arranged in a large disk at one end of the protoplast turning in a plane parallel to the surface of the leaf.

In one leaf which was treated in a 2 per cent. solution of strontium chloride for seventy-two hours (a number of cells succumb to so long a treatment in so high a per cent. of this salt) the individual lines of movement of the chloroplasts were in many cells so numerous and so indefinite in their directions that all the chloroplasts of the cell seemed to wander about aimlessly.

The fourth substance which was found to arouse the cells to abnormal activity was copper.

That copper in exceedingly minute quantities is highly toxic to protoplasm is now generally known. Nägeli did the first thorough piece of work on the poisonous effect of copper, and termed the action an "oligodynamic" one. Copper-water suitable for stimulating streaming in *Elodea* cells can best be prepared by placing several copper coins in a small quantity of water for a few days (one or two pennies in 50 c.c. of water for two days). Distilled water from a copper still will serve equally well: if too toxic it should be diluted.

Of all the reagents employed to arouse protoplasm to streaming "oligodynamic" water proved to be the most certain. *Elodea* studied in London as compared with that worked upon in Geneva was found to be much less responsive (as regards streaming) to alcohol and strontium, slightly less responsive to saponin, but equally responsive to copper.

Considerable variability in the reaction to the various reagents used was found in different cells of a leaf, in different leaves of a

plant, and in different plants collected at different times and in different localities, but never in any material did "oligodynamic" water fail to arouse a number of cells to active streaming. Many cells of the leaves of *Elodea* are stimulated to abnormal streaming, both as to rate and type, after eighteen to forty-eight hours in copper water, but many cells also succumb.

One or two of the unusual types of streaming above described may occasionally be observed in untreated cells. Consequently, all of the above types cannot be regarded as abnormal. Never, however, does an untreated cell exhibit anywhere near so great a variety of types, and never such an abundance of streaming at such an extraordinarily high rate as do cells treated in solutions of alcohol, saponin, strontium, and copper.

It is surprising that substances physically and chemically so different as are methyl alcohol, the colloid saponin, the electrolyte strontium chloride and the metal copper (it is not certain in what form copper exists when minute quantities of it go into "solution" in water—possibly as an oxide) should all stimulate protoplasm to such active and such abnormal streaming. It is further of interest to point out that calcium, a bivalent cation like strontium, does not arouse protoplasm to activity.

One naturally seeks for some cause of this stimulation to streaming and especially of the remarkable fact that such diverse substances bring about the same end result. Three possible partial explanations come to mind—a reduction in viscosity, a decrease in surface tension, and an alteration in electrical charge.

A reduction in viscosity would lessen the resistance against which the force causing streaming must act and thus permit a more rapid flow. But a reduction in viscosity would not arouse a quiescent cell to activity nor produce abnormal types of streaming. Furthermore, reduction in viscosity does not apparently always take place, indeed, an increase in protoplasmic consistency is sometimes evident.

Changes in surface tension and electrical charge have been much used of late to explain many vital phenomena. That both play an important part in living processes is probably true (although one worker would have us believe that the living substance is "electrically bland"), but many of the assumptions which have been made relative to the biological significance of these forces are highly speculative.

Alcohol, saponin, strontium, and copper all clearly and markedly reduce the surface tension of protoplasm, *i.e.* the tension existing

between the protoplast (the plasma-membrane) and the cell wall (probably the colloidal aqueous solution with which the cellulose wall is permeated). But a uniform reduction in the tension existing between the surface of a flowing liquid and the walls of the containing vessel would not alter the rate of flow.

There is another force a reduction of which might affect the rate of flow, namely, intrinsic pressure (the internal molecular force of cohesion). That a change in intrinsic pressure takes place with a change in osmotic value due to alterations in the chemical composition of the cell is inevitable. Decreased intrinsic pressure would probably mean a more rapid flow. But any statement as to the extent to which a change in the intrinsic pressure of protoplasm would affect the rate of streaming would be purely conjectural.

Streaming in protoplasm is generally regarded as a mass movement of the protoplasm *in toto*. When viewed through an oil-immersion lens the appearance of flowing protoplasm in *Elodea* cells is that of a moving rope of viscous substance. It is possible, however, that the flow may be a movement of protoplasmic particles (colloidal and microscopic) in a quiescent basic medium, *i.e.* a phenomenon comparable to cataphoresis. The one-way streaming in *Mucor* (bread-mould) very markedly resembles the phenomenon of cataphoresis. If the streaming of protoplasm is some such electrical phenomenon then an increase in the surface charge of the protoplasmic particles would possibly result in an increase of streaming.

That three of the four substances which arouse streaming are possessed of electrical properties suggests that the stimulation is an electrical phenomenon. Just what part the non-electrolyte alcohol would play in this scheme it is difficult to say, although it is evident that alcohol, and all the reagents which bring about marked changes in permeability and consequent reduction in concentration of the cell contents, must cause pronounced disturbances in the normal electrical state of the cell.

These hypotheses of the possible causes of stimulation to protoplasmic streaming are purely speculative. It is not the intention of the writer to support any one of them, but rather to indicate their weakness. Until we know something of the physics of protoplasmic streaming we cannot hope to say much on the possible causes of a stimulation to streaming.

BOTANY DEPARTMENT,
KING'S COLLEGE, LONDON.

THE NEW PHYTOLOGIST

VOL. XXI, No. 3

JUNE 1, 1922

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

III. THE STRUCTURE OF THE ENDODERMIS IN RELATION TO ITS FUNCTION

By J. H. PRIESTLEY AND EDITH E. NORTH

CONTENTS

	PAGE
Introduction	113
Preparation of the Endodermis by Maceration	114
Developmental Stages of the Endodermis	116
The Primary Endodermis	117
(1) Its Micro-chemical Structure	117
(2) The Casparian Strip	118
(a) Impregnating Substances of the Casparian Strip	120
(b) Basal Substances of the Casparian Strip	123
(3) The Function of the Primary Endodermis	124
The Secondary Endodermis	125
(1) The Suberin Lamella	125
(2) The Undulation of the Casparian Strip	131
(3) The Function of the Secondary Endodermis	132
General Discussion	135
Summary	137
References	138

INTRODUCTION

A PART from the Lycopodiaceæ (Mager⁽¹⁵⁾) the endodermis is typically present in the roots of all vascular plants and it is frequently present in the stem also. We have been led to re-investigate the structure and functions of this layer of cells as the result of a conviction that it plays a very important part in the exchange of water and solutes between the tissues on either side of it. This rôle has been assigned to the endodermis in an explanation recently advanced of the phenomena of exudation pressures in plants (Priestley⁽²⁰⁾, Priestley and Armstead⁽²²⁾).

The varying structure and function of endodermal tissue at different stages of its development has resulted in much confusion. We have therefore re-defined these stages, clearly enunciated many years ago by the Marburg school of physiological anatomists under the guidance of Prof. Arthur Meyer. This method of dealing with the subject has involved separate discussions of the structure and behaviour of the same endodermis at two different stages in its history. The general rôle of the endodermis within a plant has been discussed in a later section after the facts as to the structure, function and occurrence of the different types of endodermis have been given.

Incidentally it has been possible, thanks to new technique employed, to contribute some further facts towards an understanding of that very well-known phenomenon, the undulation of the Casparian strip. These facts have been gathered into a short section at the conclusion of the account of the micro-chemistry of the "secondary" endodermis.

Investigation of the micro-chemistry of the endodermis has so far been carried out upon the cells of this tissue as seen in section. This material was found so unsatisfactory that in the end the method was abandoned for another which provided material both for micro-chemical and macro-chemical investigation.

PREPARATION OF THE ENDODERMIS BY MACERATION

The study of the micro-chemistry of the endodermis was begun by observing the behaviour of sections towards various reagents, but this method of investigation soon raises problems it is unfitted to solve. Attempts were therefore made to isolate the endodermis from other tissues so that macro-chemical methods might be employed. The main obstacle to isolating the endodermis is the existence in the normal stem or root of three almost equally resistant tissues, cuticle (or periderm), endodermis, and xylem, all associated in a matrix of cellulose relatively easily removed by hydrolysis.

From the work of Zimmermann (35) *loc. cit.* p. 152), extended by Kroemer (13), it should be practicable to separate xylem from endodermis by treatment with eau de javelle since in a section the xylem disintegrates so much more rapidly. There appeared to be the nucleus of a method here, as it is relatively easy in many plants to pull out the vascular strands and so free them from periderm or cuticle.

Efforts in this direction have so far met with no success, presumably because the endodermal tissues are so much more exposed to the action of the macerating agent under these conditions that their disintegration is as rapid as that of the xylem strand within.

Complete success was however obtained by other means. Leafy stems of *Potamogeton perfoliatus* L., in which the endodermis is strongly developed and the xylem absent, were collected in great quantity. The plant stalks, freed from leaves, are placed under damp cloths and left for some days to decompose. They are then immersed in water, and with a little practice it is easy to extract from the partly rotted material the central endodermal cylinder, still quite firm and unaltered; light rubbing between finger and thumb removes the slight traces of cuticle remaining at the nodes. If the material is subsequently dropped into concentrated sulphuric acid, the residue is certainly more than 99 per cent. pure endodermal tissue, less hydrolysable cellulose. This tissue forms a very delicate cylinder; so long as it is in concentrated sulphuric acid its texture is so mucilaginous and fragile that it is difficult to handle, but when the acid is diluted to about 10 per cent. the cylinders become of much firmer consistency. They then float at the surface of the liquid and can easily be picked up with a bent platinum wire. They are then washed free from sulphuric acid and dried; and in this state provide material suitable for macro-chemical investigation.

By this means, during 1920, some 2-3 gms. of relatively pure endodermis were collected and some useful preliminary chemical data obtained. The further macro-chemical investigation is being pursued but will be a slow process because of the scale upon which operations have to be carried out to obtain enough of the material for analysis. In the meantime, this method of isolating endodermal tissue has provided material, in the shape of endodermal strips isolated from the rotting material, which has been of very great value for the micro-chemical studies. These strips, if allowed to rot further in water, remain coherent endodermal cylinders from the centre of which the parenchymatous tissue altogether disappears. Material prepared in this way has been widely employed and frequent reference is made to it subsequently under the term of endodermal "strips." Unfortunately the method is not readily applicable to all plants even for micro-chemical purposes where complete removal of the xylem is not necessary, and although the endodermis of other plants has been studied in this way, productive results have been obtained mainly from *Potamogeton*.

DEVELOPMENTAL STAGES OF THE ENDODERMIS

Any investigation of the endodermis at the present day naturally starts from the sound basis of facts, critically established, and published in great detail in the long series of papers issuing from the Marburg laboratory. To these papers we shall often refer in the following pages and, although we find ourselves differing from the general standpoint of this school of investigators as to the function of the endodermis, we must express our indebtedness to them for an extensive series of data, and for very valuable suggestions as to micro-chemical methods which we have employed in our own work.

In one of the earliest papers in this series Kroemer(13) points out that during its development the endodermis of the flowering plant passes through a series of stages in chronological succession. These stages differ from one another in important structural particulars, and from the standpoint of physiological anatomy must receive separate consideration.

They may be summarised as follows (Kroemer, *loc. cit.* pp. 87 *et seq.*):

1. The Embryonal Stage, where the cells are typical constituents of normal merismatic tissue.
2. The Primary Stage, in which the individual cells have relatively thin, unsubsided membranes and in which the radial and transverse walls show the characteristic Casparian strip.
3. The Secondary Stage, in which the walls are still relatively thin, but are characterised by the possession of a suberin lamella, usually deposited over the whole inner surface of the cell.
4. The Tertiary Stage, characterised by the deposition within the suberin lamella of inner lamellæ, consisting mainly of cellulose; these deposits often reach very great thickness, particularly upon the inner tangential wall.

An endodermal cylinder, in which the cells have reached the tertiary stage, may have passed in succession through all the previous stages (this appears to be always the case in the root, but not in the stem), but the primary or any succeeding stage may represent the final stage for the endodermis of various plants. Thus the work of Rumpf(26), Mager(15), and Bäsecke(1) makes it clear that in the Pteridophyta, with the exception of the Leptosporangiate Ferns, the primary stage is usually the final stage of differentiation reached in the root. In the Leptosporangiate Ferns the roots usually have as

final stage an endodermis in the secondary stage (Rumpf), similarly in the stem and frond, with the exception of *Trichomanes* and the storage rhizomes in the more complex types (Bäsecke). A tertiary stage endodermis does not appear to occur in the Pteridophyta. All these workers on the Pteridophyta quoted above have met with cases where the suberin lamella is only deposited over the inner tangential surface of the cell, and in all these cases it covers completely the whole surface up to the inner edge of the Casparian strip.

Of the four stages distinguished by Kroemer it will only be necessary to consider two in this paper. In the embryonal stage the cells of the future endodermal cylinder possess the same relation to the sap contained within them as do the cells of the apical meristem, a problem which is dealt with in the next paper in this series (Priestley and Tupper-Carey(23)).

The structure and function of the endodermis in its primary and secondary stages will now be considered at length. It will be clear after the secondary stage has been discussed that the tertiary stage requires no further attention; from our present standpoint these two stages of the endodermis may be regarded as functioning in a similar way.

THE PRIMARY ENDODERMIS

(1) *Its Micro-chemical Structure.*

At this stage the walls of the endodermal cells are so thin that it is difficult to distinguish anything beyond a thin cellulose wall, in which one band stretching around both radial and transverse walls, and frequently undulating, forms the characteristic Casparian strip (*c.s.* Text-fig. 1).

Recourse to macerating agents which do not attack cellulose will show, however, that we must distinguish between the layer most external to the protoplast, which is the middle lamella (*m.l.* Text-fig. 1), and the cellulose lamella within this (*c.l.* Text-fig. 1). When sections of herbaceous stems or roots possessing an endodermis are macerated in dilute acids followed by dilute alkalies, or by prolonged standing in ammonium oxalate after dilute acids, so that the tissues disintegrate owing to the solution of the middle lamella (Mangin(16)), then the endodermal layer is freed from the tissues adjacent to it owing to the solution of the middle lamella upon the tangential walls. The endodermal cells, however, do not separate from each other as the cementing middle lamella of the radial walls does not disappear.

Furthermore in thick macerated sections the endodermis will be found to flatten into a band in which it can be seen that cells vertically above one another also remain firmly attached, so that on both radial and transverse walls the middle lamella is untouched by these macerating fluids. We must now consider how the different behaviour of the middle lamella in these regions is related to the presence of the Casparian strip.

(2) *The Casparian Strip.*

Kroemer (*loc. cit.* pp. 90-94) discusses the nature of the Casparian strip very fully. He concludes that the strip is formed of substances different from those composing the rest of the wall, and which are insoluble in concentrated sulphuric acid. These substances after

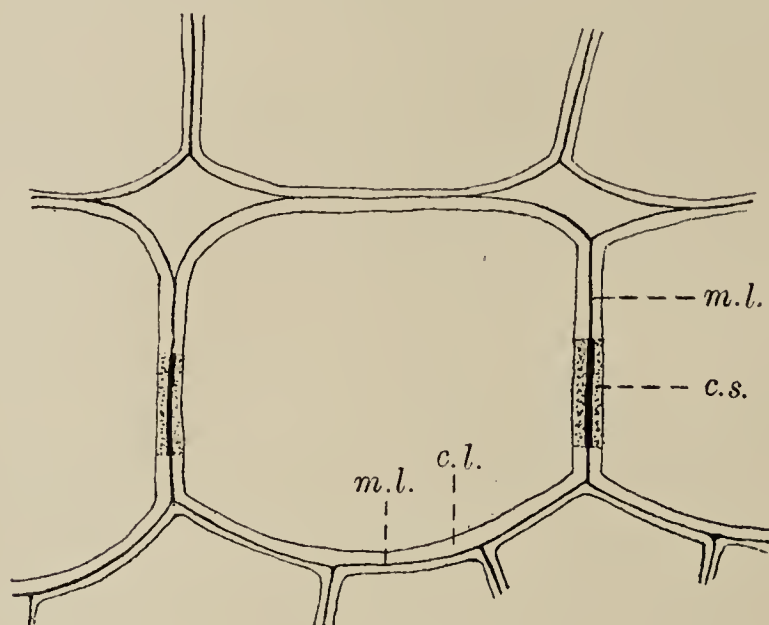


Fig. 1.

treatment with eau de javelle are so altered that they are now more soluble in concentrated sulphuric acid than the remaining (cellulose) portion of the cell wall.

Apart from the Casparian strip, the whole cell wall, both middle lamella and internal cellulose lamella, is directly soluble in sulphuric acid. If, therefore, sections of the young roots are placed in strong sulphuric acid the tangential walls completely disappear, so that the transverse appearance of the endodermis in section is as shown in Text-fig. 2 (*b*). All that is left are the Casparian strips, forming thin transparent wavy bands, staining yellow in iodine, very difficult to see in section, usually appearing either as a flattened ribbon with occasional pleats as seen from the side, or a dark triple line as seen

on edge (see Text-fig. 2 (b)). The triple line appearance is also very striking in strips of *Potamogeton* endodermis after treatment with sulphuric acid. Here the groove is 1μ deep at least; and at different foci either the central line or the two marginal lines are sharply defined (see Text-fig. 2 (c)).

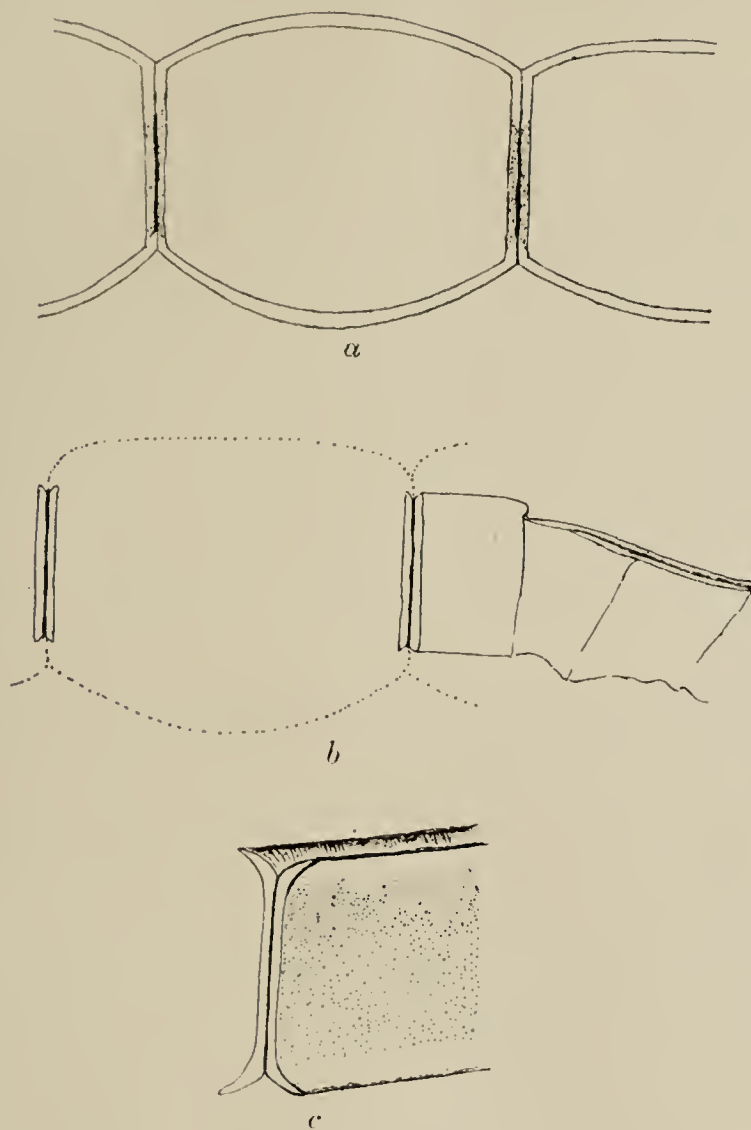


Fig. 2.

The relations of the Casparian strip to the rest of the wall must now be discussed. Kroemer's results already quoted suggest that it consists of a basal substance easily soluble in sulphuric acid, and an impregnating substance resisting sulphuric acid but dissolving in an oxidising agent like eau de javelle. Kroemer also points out that the unaltered strip gives many of the reactions of lignified membranes (red with phloroglucin and hydrochloric acid, etc.), and in its behaviour to Sudan III glycerine and to other fatty stains resembles

more nearly a lignified than a cutinised or suberised membrane. These facts are of vital importance from the physiological standpoint and have been very fully re-examined. The reactions of the strip certainly suggest the presence of basal substances which have later been impregnated with other substances, but the problem appears to be more complicated than is indicated by Kroemer.

(a) *Impregnating Substances of the Casparian Strip.*

Evidence as to the nature of these substances may be obtained from the behaviour of the Casparian strip to staining reagents and by micro-chemical methods. This evidence has been admirably presented by Kroemer (*loc. cit.* pp. 91-94) and may be summarised as follows. The staining reactions of the strip correspond very closely with those of lignified membranes; some of the reactions of suberised membranes are also given, but not more strongly than by many lignified membranes. Thus the Casparian strip stains with Sudan III but not more strongly than the xylem. The staining substances are easily removed by oxidising agents, eau de javelle, chromic acid, etc., less easily by boiling alkali; they are resistant to concentrated pure sulphuric acid and render the whole strip resistant to this acid.

From the micro-chemical examination of sections, it would therefore appear that the strip is impregnated with lignin-like substances. We conclude, however, on the basis of further experiments, that the characteristic feature of the strip is rather the presence within it of derivatives of fats or fatty acids.

Attention must be drawn to the conditions under which the strip is invariably formed. It appears in the layer of cells around the plerome cylinder, a layer which intervenes between the plerome and the first air spaces of the cortex. Within the plerome, at the level at which the Casparian strip is appearing, xylem and phloem are just beginning to differentiate. This differentiation involves great internal alteration in the future conducting elements and undoubtedly these alterations are accompanied by the giving up of organic solutes to the sap which may be assumed to percolate through the intervening walls up to the endodermal cylinder. At the outer surface of the cylinder this sap meets the air diffusing inwards from the intercellular spaces; thus we have the conditions arising which at the surface of the plant give at one time cutin, at another suberin. It is pointed out elsewhere (Priestley and Woffenden(24), quoting in particular Tison(29) and Devaux(8)), that the two processes of lignification and suberisation

frequently appear thus associated, as in the leaf scar, and that the formation of suberin is always dependent upon the presence of oxygen.

The observation of Kroemer (*loc. cit.* p. 94) and still more that of Rumpf has already associated the appearance of the Casparian strip with the differentiation of the phloem. We would suggest that this association is causal, and that the Casparian strip is impregnated with substances diffusing from the differentiating vascular strands. That these substances are in part of a fatty nature is to be expected, such fatty acids forming, according to Hansteen Cranner(5, 6), an invariable accompaniment of the membranes of parenchymatous tissues; Czapek(7) has shown also that they are abundant in merismatic protoplasts. They would certainly be lost from the contents of the developing xylem elements. When compared with the cases of cutin and suberin formation which are better known, notably the formation of suberin at the surface of wounds (Priestley and Woffenden(24)), the presence of oxidation products of fatty acids may be anticipated in the endodermal wall, and these will have the general properties of suberogenic or cutinogenic acids (Priestley(21)).

It is very difficult to give experimental evidence of the existence of these substances, because micro-chemical data are so indefinite, and macro-chemical investigations upon an isolated *primary* endodermis have not yet proved practicable. We have tried the effect of extracting the vascular strands from the petioles of species of *Plantago* and leaving them exposed to air, or immersed in water for some days. After immersion in water for a week the surface of the strand is very little altered, but if exposed in saturated air, either in the light or dark, within a week it is brown in colour and when tested with Sudan glycerine gives indications of the deposit of a substance with the staining properties of suberin upon the whole surface of the cell membranes at the outside of the strand.

In view of the physiological evidence to be presented later, showing that the radial and transverse walls of endodermal cells appear to be only slightly permeable to water and solute and thus have properties different from lignified membranes, together with the arguments presented above, we conclude provisionally that the balance of argument is in favour of the presence in the Casparian strip of oxidation or condensation products of fatty acids of the same general type as those found in suberin and cutin.

The experimental work described in this paper was completed by August 1921, but some later investigations have provided evidence,

very convincing although indirect, of the presence of fatty substances in the Casparian strip.

For some time the hypothesis had been entertained that these fatty substances were the condensation products of unsaturated fatty acids, whilst the staining reaction with dimethylaminoazobenzene, described later (p. 127), suggested that these substances remained acid in reaction.

An examination of the fatty acids present in the germinating seeds of *Vicia Faba* shows the presence of two unsaturated acids, oleic and linoleic, but not in sufficient quantity to condense readily to a solid product as in a drying oil, so that their accumulation in the Casparian strip was not accounted for.

Fortunately, at this point, attention was directed to the work of Crocker and his colleagues (*Botanical Gazette*—references will be given in a separate communication) upon the toxic action of illuminating gas or smoke from smouldering organic substances, upon etiolated pea seedlings.

In a later paper of this series (Priestley and Ewing, vi), it will be shown that the stems of etiolated pea seedlings contain a functional primary endodermis, absent from the stems of the normal seedlings. The description given of the effects produced by the toxic agent immediately suggested that the endodermis failed to form in the presence of the toxic constituent of the gas or vapour and that this structural change was mainly responsible for the visible effects noted such as increase in stem girth, irregular stem curvatures, etc.

A repetition of Crocker's experiments has confirmed this suggestion. Crocker supplies convincing evidence that the toxic substances are the unsaturated hydrocarbons in the gas, such as ethylene. It therefore appears probable that the presence of the ethylene prevents the normal accumulation of unsaturated fatty acids in the region potentially capable of forming a Casparian strip. The reason might obviously be because some constituent of the plant membrane, that normally collects the unsaturated fatty acid, is instead reacting with the ethylene gas. Ethylene could only react by virtue of the unsaturated linkage it contains, presumably therefore in the absence of the ethylene the unsaturated fatty acid is held at this point by reaction with its similar $\text{CH}=\text{CH}$ linkage. In this case it is easy to understand how the unsaturated acid becomes sufficiently concentrated in this region to condense to a hard resistant varnish in the presence of oxygen and why the unsaturated acids in this strip retain their acid reaction.

Further evidence in support of this explanation is supplied by

the fact that if root tips are soaked in an alcoholic solution of carotin, the yellow pigment is held by the wall behind the growing apex in the region of the future endodermis. Carotin is a hydrocarbon offering no other possibility of chemical reactivity than the unsaturated $\text{CH} = \text{CH}$ linkage.

(b) Basal Substances of the Casparian Strip.

No conclusive evidence exists as to the nature of these substances, but we agree with Kroemer that cellulose does not enter into their composition. If the impregnating substances are removed by oxidising agents such as eau de javelle, or by boiling alkali, then the subsequent behaviour of the section in iodine reagents shows clearly the absence of any cellulose matrix from the Casparian strip. The basal substance present is far more resistant to concentrated sulphuric acid and less resistant to oxidising agents like eau de javelle than the cellulose membranes surrounding it. It is quite possible to obtain isolated networks of Casparian strip by submitting the section or tissue, first to prolonged boiling in strong potash and then, after washing, transferring to concentrated sulphuric acid. Unfortunately endodermal tissue is usually entangled with xylem and cuticle or exodermis, and the isolation of the Casparian strip for more thorough investigation is difficult. It can be completely isolated in the way described above in the case of *Potamogeton* and therefore in time macro-chemical evidence of its nature may be forthcoming.

Preliminary observations have supplied the facts that nitrogen is present (about 4 per cent.) and also sulphur. (We have to thank Mr H. D. Kay of the Department of Physiology, University of Leeds, for his help with the macro-chemical work.) These results, together with the great resistance of this substance to sulphuric acid, show clearly that we are dealing with something which is quite definitely not cellulose nor a pure carbohydrate, but its actual nature is as yet only conjectural. We would, however, emphasise the facts that the Casparian strip is formed in the root at a very early stage, and that its formation probably involves the deposition of the impregnating substances in a wall which has not yet lost the characters of the walls of meristematic tissue, which do not at this stage readily give cellulose reactions. The nature of these walls is described somewhat more fully in the next paper of this series, where it is shown that the cell wall retains the embryonic character for a longer time at the root apex than in the stem apex of the plant. Provisionally we would emphasise the significance of the fact in explaining the constant occurrence of a Casparian strip and a primary

endodermis at an early stage in root development. In stems, on the other hand, except in submerged aquatic plants and in certain cases to be considered in later papers, notably the case of etiolated plants, the embryonic nature of the wall disappears in earlier stages at the growing point, its impregnation does not therefore occur, the fatty substances being presumably swept onward by the sap to the cuticle; as a consequence the primary endodermis with Casparian strip is never developed. In such stems a functional endodermis may never be present (Mylius(18) shows how frequently this is the case in the axis of the flowering plant aboveground), or a secondary endodermis may arise later in development, *e.g.* in *Escallonia*, *Camellia*, etc.

Summarising the results of the micro-chemical study of the primary endodermis, the cells at this stage possess a cellulose inner lamella, and an outer middle lamella soluble, after treatment with acids, in the usual pectin solvents. This wall is interrupted by a continuous band around its radial and transverse walls, the Casparian strip, which differs throughout its substance from the rest of the wall. The Casparian strip is laid down at an early stage of development before cellulose can be detected in the endodermal cell walls. In this region the original lamellæ are impregnated with complex substances which invariably contain derivatives of fatty substances, probably allied to the suberogenic and cutinogenic acids, and with substances giving reactions for lignin. The basal substance of the Casparian strip is therefore apparently closely allied to the normal wall of the merismatic cell; this basal substance contains some nitrogen, is very resistant to acids and alkalis and dissolves in oxidising agents.

(3) *The Function of the Primary Endodermis.*

Earlier work upon this subject has been summarised elsewhere (Priestley (20)), when it was shown that the experiments of de Vries(30) and de Lavison(14) suggested that the passage of water and solutes through the endodermis was considerably impeded by the structure of the radial and transverse walls, and as a consequence took place mainly, if not entirely, through the protoplasts themselves. Further experiments in this laboratory have confirmed this conclusion. An extended investigation by Miss Helen Heaps, M.Sc. on the entry of iron salts into the plant has shown that, by the use of suitable micro-chemical methods, the iron can in certain cases be traced across the endodermis, but in this case it is always found within the protoplasts

(these results are in accordance with those recently published by Ziegenspeck(36)).

Experiments designed to test the resistance of the apical meristem of the root to positive hydrostatic pressures (Priestley and Tupper-Carey(23)) have shown at the same time that no leakage takes place across the endodermis under such pressure.

As the result of experiments with acid and basic dyes, in which these dyes were driven under pressure through the xylem, the following point emerges which has probably considerable significance. In the slightly tuberous roots of *Ranunculus Ficaria* and the basal internodes of the stems of *Vicia Faba* L. basic dyes, methyl green, neutral red and malachite green were strictly retained within the endodermal cylinder, only escaping into the cortex where gaps occurred in the endodermis, as occasionally in the basal internodes of the stem of *Vicia Faba* L., or after prolonged experiment when it was clear that the dye was penetrating dead cells. On the other hand, acid dyes (acid green and eosin) penetrated the endodermal protoplasts relatively easily and thus escaped into the cortex.

These results, supported by a number of other observations, lead to the following conclusions. The primary endodermal cylinder possesses in the Casparian strip a network of wall intervening between the protoplasts which renders the radial passage of water or solutes by way of the wall uniformly difficult. There are no grounds for assuming, as is done by the whole Marburg school of investigators, that whilst impermeable to solutes the wall is readily permeable to water, and the generalisations of Ziegenspeck(36) are largely based upon experimental studies of membranes that do not possess Casparian strips. The Casparian strip therefore places the exchange of water and solute through the primary endodermal cylinder under the control of the living protoplasts. These protoplasts seem to be relatively easily penetrated by complex ions carrying an electro-negative charge, but refuse passage to complex ions with an electro-positive charge so long as they retain their living semi-permeable plasma membranes. The consequences of this remarkable behaviour of the primary endodermis will require much further elucidation, and some possible indications of its significance are submitted in later papers (Priestley and Tupper-Carey(23), Priestley and Woffenden(24)).

THE SECONDARY ENDODERMIS

(1) *The Suberin Lamella.*

The essential structural difference between the primary and secondary stages of the endodermis is that, in the secondary stage

a suberin lamella is deposited usually over the whole internal surface of the cell wall, including the Casparian strip, and lining every pit in the original membrane. The only new micro-chemical question that falls to be discussed therefore is the nature of the suberin lamella. In practice, this again proves a very complicated problem.

In the first place are we justified in calling it a suberin lamella? Von Höhnelt(11) first showed that substances of a corky nature were present in the endodermis in its secondary stage. He regarded these substances, found in periderm and endodermis, as one characteristic substance, suberin. In the endodermis he identified it by two reactions he regarded as characteristic, viz. (1) The cerin or cerinic acid reaction, obtained on treating the layer with strong oxidising agents such as Schulze's macerating fluid (potassium chlorate in concentrated nitric acid). This reaction is given very beautifully by strips of *Potamogeton* endodermis, especially if the cellulose is first removed with concentrated sulphuric acid. On gently warming the endodermis in the macerating fluid, the wavy walls collapse and give rise to yellow spherical drops, solidifying on cooling and soluble in fatty solvents. (2) The reaction with caustic potash. Warmed with the reagent three layers can be distinguished in the cell membranes, as in the case of periderm cells. These are, outermost the middle lamella, which on warming is recognised as a dark wavy line on either side of which can be seen the suberin lamella, and within this again von Höhnelt noted the cellulose layer of the tertiary stage.

The development of our knowledge of suberin since the days of von Höhnelt has been traced elsewhere (Priestley(21)). We have re-examined the question in the light of this more recent work and conclude that there is evidence (1) that the "suberin lamella" is impregnated with condensation products of bodies like suberogenic acids, but not including the phellonic acid characteristic of the suberin of the cork oak; (2) that these products of fatty acids impregnate a basis which is not cellulose nor does it seem to be identical with the basal substances of the Casparian strip.

Our evidence for these statements is as follows:

(a) *Evidence for "suberogenic acids."* Using Van Wisselingh's methods(33) sections of various roots were carefully warmed in concentrated potash over a micro-burner. With periderm tissue the fats in the wall readily melted out into large balls, but in sections of roots the successful production of these balls was very difficult, probably because of the small amount of membrane under examination. More success was obtained using the method with strips of

Potamogeton endodermis. The balls thus obtained, with iodine and potassium iodide and dilute sulphuric acid, or with chlorzinc-iodine, stained dark brown or reddish brown but never the colour of the similar balls obtained from periderm, which turn red violet in colour owing to the potassium phellonate they contain.

Strips of *Potamogeton* endodermis were then heated in anhydrous glycerine. Fusible products were obtained at 280° C., the temperature at which Van Wisselingh obtained similar results in the case of the periderm of *Salix caprea* and *Ilex aquifolia*. On heating in glycerine, after three days maceration in 50 per cent. potash, the contents fused out of the suberin lamellæ at a temperature of 95°–100° C. The balls of potassium soaps of organic acids obtained by these methods never gave the phellonic acid reaction with iodine reagents. The absence of phellonic acid was further confirmed by boiling considerable quantities of the *Potamogeton* strips upon a microscope slide first in potash and then in alcohol. The potassium soaps formed are soluble in alcohol and as the alcohol gradually boils away are left concentrated in one small portion of the preparation. The relatively large concentration of fatty substances thus obtained gave no phellonate reactions.

Finally, using the methods of Gilson (9), a considerable bulk of the endodermis was boiled with alcoholic potash. On concentrating the solution thus obtained, a small quantity of a crystalline precipitate formed on cooling. This precipitate did not give the reactions of phellonic acid, and its further characterisation awaits fuller macrochemical investigation.

The results obtained thus far show that the suberin lamella undoubtedly contains substances of the general type of the suberogenic acids, but in no case so far examined has phellonic acid been found amongst these acids. In view of this conclusion it is important to note that we can confirm Kroemer (13) and Mylius (18) as to the distinctly different behaviour to various reagents of suberin lamella and Casparian strips. Phloroglucin (or catechin) followed by hydrochloric acid stains the Casparian strip red and the suberin lamella yellow, thus indicating that the suberin layer, unlike the Casparian strip, is not always impregnated with substances giving reactions characteristic of lignin.

Dimethylaminoazobenzene (in an alcohol glycerine solution) followed by concentrated hydrochloric acid stains preparations which have previously been soaked in 1 in 300 hydrochloric acid, the Casparian strip becoming red, the suberin layer yellow. This reaction was obtained most beautifully with the rhizome of *Pteridium*

aquilinum L. after brief previous treatment of the section with eau de javelle, the yellow suberin layer, present on the inner tangential wall only, standing out very clearly because it had separated from the lamella exterior to it. The colour reactions here obtained indicate that the Casparian strip is more acid in reaction than the suberin lamella. That the strip is acid and the suberin lamella neutral in reaction is also suggested by their reaction with nile blue, when the Casparian strip is stained deep blue and the suberin lamella violet to pink.

(b) *Evidence that the basis of the suberin lamella is not identical with that of the Casparian strip.* This evidence is obtained mainly from examination of the tertiary stage in *Potamogeton* strips. It is

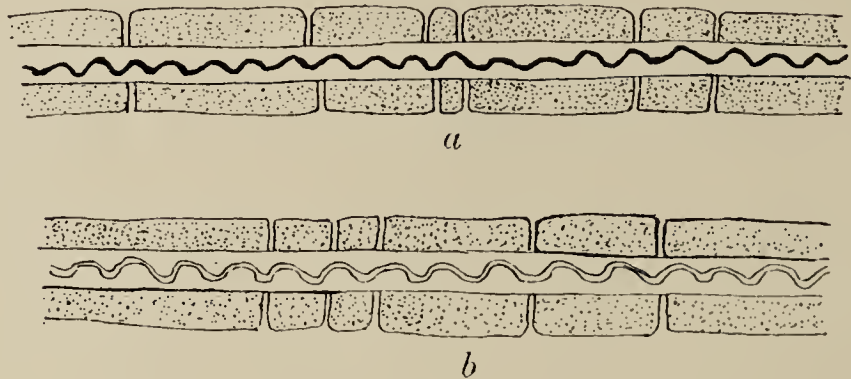


Fig. 3

difficult to obtain because the lamellæ are very firmly cemented together. In the first place prolonged maceration, lasting many days, seems to dissolve slowly the cementing material which is presumably some of the deposited fatty acid products. If the tertiary endodermal strips, after short maceration or boiling in concentrated potash, are mounted in iodine and sulphuric acid the layers described by von Höhnelt are seen very clearly. In the joint wall between two cells is seen the dark wavy line of the middle lamella, outside this the suberin layers, and beyond these again the strongly pitted cellulose layers (Text-fig. 3 (a)). At a focus about 1μ or 1.5μ higher the dark middle lamella is no longer seen, in its place is a wider wavy strip of yellowish or greenish tinge (Text-fig. 3 (b)). On prolonged maceration this strip separates from the cells on either side and then often loses its wavy appearance. This separation can most readily be produced by mounting the strips, after washing them in water on removal from the strong potash, in concentrated hydrochloric acid and gently tapping the cover glass. The strip then separates in many places from the

adjoining cells, and straightens out in the manner shown in Text-fig. 4. This separated strip is clearly the normal Casparian strip and under various treatments can be shown to possess its characteristic appearance and properties, as already described and figured (Text-fig. 2). But the separated cells still have their inner thick cellulose membrane surrounded by a continuous suberin lamella, staining brown with iodine reagents, so that by this method we have separated the Casparian strip from the suberin lamella.

The difference between Casparian strip and suberin lamella is shown by their behaviour towards oxidising agents, and towards acids and alkalis. The Casparian strip is much more susceptible to the action of oxidising agents. Chromic acid or Schulze's macerating fluid completely dissolves it in a few hours, leaving the long endodermal cells of the *Potamogeton* strips isolated in the macerating fluid. If the reaction is stopped in time, on treatment with iodine reagents, the suberin lamella can still be seen surrounding these isolated cells. Eau de javelle dissolves the Casparian strip more slowly but it disappears after three or four days from between some of the cells, and in other parts of the strip it appears thin and worn into holes, whilst the suberin lamella remains unaffected.

On the other hand, the Casparian strip, as already shown, is extremely resistant to strong acids and alkalis. If the strips of tertiary endodermis are treated first with boiling concentrated potash, the suberogenic acids are removed from both the Casparian strip and the suberin lamella, leaving the Casparian strip regularly undulated and contracted as already described. But some basal substances must be left to the suberin lamella as the Casparian strip remains firmly attached to the inner cellulose wall, and the undulations of the Casparian strip are regular and of small amplitude. On now transferring the strip to sulphuric acid after washing in water, the cellulose walls dissolve and also the remaining basal substance of the suberin lamellæ, and the Casparian strip alone is left, much swollen and waved in the wildest fashion (see Text-fig. 5). These experiments seem conclusive evidence that there is a basal substance to the suberin lamella and that it is different from the basal substance in the Casparian strip, and thus we are presented with another unknown substance or class of substances.

Provisionally it is suggested that it will be well to regard the basal substances of the suberin lamellæ as somewhat resistant carbohydrates, akin to the inner carbohydrate lamellæ of the tertiary stage. The work with *Potamogeton* has shown that these inner

lamellæ though giving most of the reactions of cellulose cannot be classed as pure cellulose in this species as they cannot be dissolved under any conditions by a solution of zinc chloride dissolved in twice

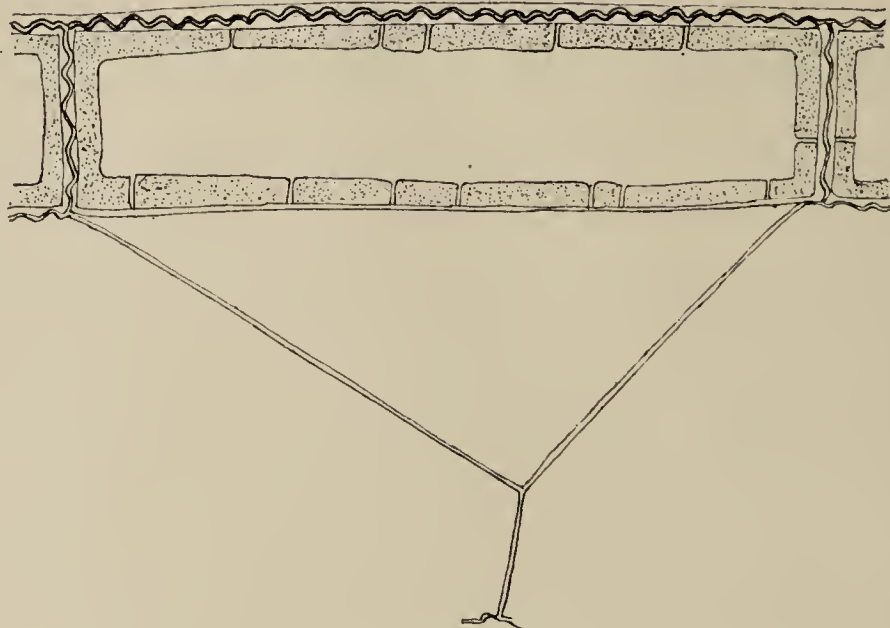


Fig. 4



Fig. 5

its weight of concentrated hydrochloric acid or by ammoniacal cupric oxide (Schweizer's reagent). The existing state of our knowledge of the chemistry of cellulose hardly permits of a profitable prosecution of this point at present, but the existence of a series of more resistant bodies, possibly anhydride derivatives allied to cellulose, seems more than probable, especially in association with suberin (Zemplen (34)).

For the present we may summarise as follows our conclusions with regard to the suberin lamella characteristic of the secondary stage of the endodermis. The lamella has a basis, differing from the basal substance of the Casparian strip in its ready solubility in sulphuric acid; it may possibly be a more resistant cellulose derivative chemically allied to the substances present in the thickening lamellæ of the tertiary stage. This basal substance is impregnated with derivatives of suberogenic acids forming a product like suberin, but differing from typical suberin in that phellonic acid cannot be identified amongst the constituent acids.

(2) *The Undulation of the Casparian Strip.*

This phenomenon, frequently discussed by earlier observers, has been re-investigated during the micro-chemical study of the strips of *Potamogeton* endodermis. Schwendener (27) regarded the undulation as due to the disturbing effect of section-cutting upon the turgid tissues of the root. The cellulose walls being elastic contracted when the tension was released, whilst the Casparian strip, unable to contract, was thrown into folds. Van Wisselingh (32) agreed that in some cases the undulations first appeared in the section but pointed out that in other cases they could be seen in the living root. Like Strasburger (28) he regarded the undulations as due to more rapid local growth of the wall during suberisation. Rimbach (25) confirmed Van Wisselingh's observation that the undulations are present in the living uncut root. He also showed that they may be produced (i) by plasmolysing the living endodermal cells with the aid of salt solutions; on again transferring the tissues to water, the strips straightened out as the cells became turgid; (ii) as a result of the general contraction of the root after it had reached its maximum length in growth. In this case the undulations would occur in the suberised layers of the secondary or tertiary stage of the endodermis.

In our observations upon the endodermal strips of *Potamogeton* it was always found that the walls of the cells of the endodermal cylinder, after maceration in water, were quite straight whether in the primary, secondary, or tertiary stage, but the Casparian strip could be thrown into the very characteristic folds by treatment either with potash or sulphuric acid.

The employment of these strips made it readily possible to ascertain whether the formation of these folds was connected with cell contraction or expansion. In some cases the strips were placed on microscope slides over glass scales ruled in millimetres, in other

cases the length of the strips was measured under the microscope by the use of a cross wire in the eye-piece and a mechanical stage with a micrometer screw traverse. In concentrated sulphuric acid observation showed that the whole strip increased somewhat in its length. As the Casparian strip was thrown into a series of slight regular folds, the conclusion is inevitable that the increase in length of the Casparian strip as a result of swelling was greater than that of the suberin lamellæ which enclosed and restrained it.

In cold concentrated potash the membranes were unaffected but on warming a marked undulation was produced and the strips shrink in length by a very appreciable amount (5–20 per cent. of their original length). This suggests that the inner cellulose lamellæ of the tertiary *Potamogeton* endodermis contract most and carry with them the Casparian strip, which contracts less and is therefore thrown into folds.

If endodermal strips thus treated are now washed and transferred to concentrated sulphuric acid, cellulose walls and the basal substance of the suberin lamella both dissolve, the Casparian strip is no longer restrained from showing the full swelling effect of the acid and a wildly irregular wavy outline is the result (Text-fig. 5).

(3) *The Function of the Secondary Endodermis.*

In many cases the cylinder of an endodermis in the secondary stage contains isolated cells, more rarely groups of two or three cells vertically above one another (Mylius(18)), which remain in the primary stage. These are the passage cells. Very rarely, as in *Spiraea ulmaria*, the numbers of cells remaining in the primary stage is exceedingly high; Mylius gives it as 45 per cent., a more usual proportion is one cell in eight or ten. Frequently, especially in the secondary and tertiary endodermis of roots, there are no passage cells and the secondary endodermis is completely closed until later it may be broken by internal growth activities as described by Mann(17) for *Dracæna fruticosa* Koch. If passage cells are present they will function in the manner already described for the primary endodermal cells.

If no passage cells are present, the physiological properties of the secondary endodermis will depend upon the behaviour of the suberin lamella to water and solutes. This subject is very fully discussed by Mylius(18) where earlier work is fully dealt with. It is difficult to see how Mylius reaches his conclusion that the suberin lamella, whilst impeding the entrance of water and solutes, must let them pass; but apparently he bases it chiefly upon the fact that he has confirmed

the earlier observation of Schnee that the protoplasm within the suberin lamella will plasmolyse, although slowly. He therefore concludes that the protoplast within the suberin lamella remains alive for many years in perennial tissues and its surrounding suberin lamella must therefore remain permeable to water and the dissolved substances necessary to support life.

Osterhout (19) has shown that plasmolysis is a good criterion of the vitality of a protoplast but he has also shown that there are many states of contracted protoplasm which must be distinguished from true plasmolysis. We do not feel competent to challenge Mylius's observations upon plasmolysis from our more limited series of observations but would emphasise the fact that the endodermal protoplast will contract away from the wall under a most varied series of reagents, ranging from plasmolysing agents to strong acids. In all such cases it appears as a band stretching across the cell and is invariably attached to the Casparian strip. We have no confidence that in any case could this appearance be ascribed to a true process of plasmolysis, neither is it any evidence of the penetration of the suberin lamella by any of these reagents when acting upon an endodermis in an uncut plant.

On the other hand, there is convincing evidence that the cells of the secondary endodermis are completely impermeable to toxic electrolytes in strong concentrations which readily penetrate protoplasmic membranes if they can achieve contact with them. This evidence is a strong argument in favour of the conclusion that the suberin lamella is impermeable to all inorganic solutes. Mylius quotes the experiments of Schwendener on the penetrability of secondary and tertiary stages of the endodermis, which are really tests of the permeability of the suberin lamella. These experiments are not free from objection owing to the reagents employed. Thus in the experiments in which he looked for inward diffusion through the endodermis of iodine from various aqueous solutions, it must be remembered that the suberin lamella is impregnated with fatty substances which dissolve iodine, so that its slow penetration is probably due to its solubility in the lamella. The use of tannin is not open to this objection and after tannin had been sucked through the rhizome of *Iris germanica* for a day sections of the rhizome placed in iron salts showed no trace of tannin in the cells of the endodermal cylinder or cortex. In similar experiments with the roots of *Iris germanica* Schwendener found that the tannin passed out through the endodermis by the passage cells only. Tannin is such an active precipitant that although the above experiments are very convincing, it might

be argued that its obstructed passage was due to its own precipitating qualities. Similar experiments were therefore carried out with other salts, using large Monocotyledonous roots having a tertiary stage endodermis without passage cells, also the rhizome and fronds of various Polypodiaceæ where the secondary endodermis was unbroken by passage cells. In some of these experiments lead, iron and copper salts in strong concentration were driven up the vascular strands under pressure, and subsequent micro-chemical tests showed that the cells within the endodermis were completely penetrated but none of the toxic salts were present in the cells of the endodermal ring or outside it. In other experiments the toxic salts were allowed to diffuse in through the exposed cortex, and the endodermis then proved a barrier to their inward diffusion.

It is difficult to perform critical experiments upon the permeability of the suberin lamella to water, but the resistance of the suberin lamellæ of the periderm to evaporation is almost an axiom among botanists. Mylius summarises what evidence there is to show the impenetrability of the dead suberin lamella in the periderm to gases, a property which, in view of the gas enclosed within the dead cells, would give the cork layer good insulating properties. Haberlandt (10) (*loc. cit.* p. 137) gives experimental evidence for the general reduction in evaporation produced by the cork layer as a whole. As differences have definitely been established between the suberin lamella in the endodermis and this lamella in the periderm, it is not possible to take over these experimental results bodily, but a general similarity of behaviour of the two lamellæ may be expected. This expectation seems to be borne out by the result of the following experiment. Some lengths of the thick prop roots of a Palm were taken, and the thick impermeable outer layer removed. These roots possess an endodermis in the tertiary stage without passage cells. The lengths of roots were divided into two groups, in one group the cut ends were sealed in paraffin wax, whilst the ends of the other group were left unsealed. Both groups were weighed, then placed in a desiccator over calcium chloride and weighed again at intervals. The unwaxed pieces lost weight most rapidly and in a few days reached a constant weight, the waxed pieces still lost weight slowly for another week and then were cut in half transversely and left to reach a constant weight also. The exposed end of the waxed pieces were then sealed up and all the pieces transferred to water and left for nearly two weeks. At the end of this time the unwaxed pieces were within 0.2 gm. of their original weight, whilst the waxed pieces were still 1 gm. (about 10 per cent.) short of their original weight. This

experiment is not conclusive but it certainly suggests that the endodermal cylinder, with its suberin lamellæ, offers a considerable resistance to the ingress and egress of water.

GENERAL DISCUSSION

The general consequences of the view taken in this paper of the functions of the primary and secondary endodermis are very far-reaching. Here it is only possible to emphasise the following points:

In the absorptive region of the root a primary endodermis is present in all vascular plants with the exception of the Lycopodiaceæ. A consideration of the previous pages will show that this primary endodermis possesses all the qualities essential for the development of sap pressures, as enumerated in a previous paper. Whilst many points in the complex micro-chemistry of the cell membranes remain to be cleared up, it is hoped that this general conclusion is soundly established.

On the other hand when, as in the older roots and stems of many Angiosperms and the Filices, an endodermis of secondary or tertiary stage develops, an entirely new situation arises. If this endodermis contains no passage cells, then the vascular strands it encloses must be regarded as solely conducting in function. The full consequence of this view, when applied to the Filices requires fuller consideration than it has yet received. As a result of our own observations together with a consideration of Bäsecke's fundamental researches, we reach the conclusion that in many Ferns the stele throughout the greater part of its passage in rhizome and frond is practically isolated from its surrounding cortical tissues. Bäsecke's experiments upon the movement of starch from the partly shaded leaves of *Scolopendrium* supply good evidence that it can only pass from the lamina into the vascular strand in the region of the sorus, where alone the endodermis is not in the secondary stage. We note also his remarkable observation that in every fern frond of the Filicineæ that he could examine growing in its native country, when the sori were mature the vascular strands in the leaf become completely isolated from the lamina by the completion of the secondary stage of the endodermis throughout the course of the vascular bundle. The subsequent death of the lamina Bäsecke then attributes to lack of solute, and we would add, to lack of water also.

With the help of Miss Armstead, M.Sc., some preliminary experiments have been carried out upon the changes in the cell membranes in the cortical tissues of the Filicineæ. As a result the conclusion is reached that the "vagin" of Bäsecke is only a form of the phlobo-

tannin isolated from the cortical tissue by Walter (31). The brown substance present in the membrane of the cortex of frond and rhizome of *Pteridium aquilinum* L. appears to be a phlobaphene arising from a phlobo-tannin or catechol-tannin, which can be detected in the young fronds by micro-chemical tests, but which is completely absent from the mature fronds. Substances of this nature are frequently formed from cellulose or other carbohydrate derivatives, as is shown by the work of Hoppe-Seyler (12), Conrad and Gutzheit (4), and Beckley (2).

As Hoppe-Seyler points out these substances usually only arise in dead tissues and he questions the identification with these "humin" substances, of phlobaphenes appearing in living tissues. But in great tracts of the cortical tissue of the Filicineæ we have a tissue which, if not dead, is dormant, and isolated from the water and solute supply in the vascular tract by a most obstructive endodermis. The cells as a consequence lack vitality, undergo considerable desiccation and processes are initiated in the ground tissues which normally await the death of the plant. Thus phlobaphenes, probably anhydride derivatives of catechol-tannins, are formed and deposited in the cell membranes and probably contribute towards making these membranes relatively impermeable.

It is a consequence of this view that the starch stored in the mature rhizome of a plant like *Pteridium aquilinum* L. would seem to be lost to the plant. We have searched the literature, so far in vain, for a description of its subsequent utilisation, and Miss Vera Hunter, M.Sc., has made preliminary examination of the rhizome during the growth of the leaves in the spring of 1921 without detecting any signs of its removal. It may be slowly accessible by cortical diffusion to the growing regions but this seems to be unlikely.

The application of these considerations to phylogeny we leave to others, but we would point out that Bower's (3) interesting contribution needs revision in the light of the fact that he assumes an exchange under protoplasmic control between cortex and vascular strand in plants where the secondary stage of the endodermis is present. The general validity of the argument as to the relation between stelar bulk and the need for surface exchange of gases and nutrient materials may not be affected by such a revision. Such an exchange is only essential, probably, so long as the stele is developing, and in the short developmental region of the rhizome or frond the endodermis is in the embryonal or primary stage.

Many problems of great intrinsic interest await the application to the Angiosperms of these considerations as to endodermal function and structure. Some of these problems are discussed in subsequent

papers of this series, others are still under investigation. We hope, however, that this discussion has proceeded far enough to show the wide interest of the botanical questions involved.

SUMMARY

1. This paper presents an attempt to re-investigate the micro-chemistry and structure of the endodermis to see how far the facts support the view recently advanced as to its function in the plant.

2. Until now micro-chemical studies of the endodermis have been made mainly on sections of tissues. A method is described of using cylindrical strips of endodermis from *Potamogeton perfoliatus* L. which has been found much more convenient.

3. At the outset, certain stages in endodermal development are defined, as they need separate consideration both as regards structure and function.

4. The embryonal stage is not discussed, as the cells have the properties characteristic of those of the apical meristem, discussed in another paper of this series.

5. The primary stage is characterised by the presence of the Casparian strip. The rest of the wall is at this stage cellulose bounded externally by a normal middle lamella.

6. The Casparian strip consists of impregnating substances of various kinds, including derivatives of fatty acids and substances giving the reactions characteristic of lignified membranes. These are embedded in a basal wall substance which is of unknown composition.

7. The unknown basal substance present in the Casparian strip is characterised by its great resistance to concentrated acids and alkalis; it contains some nitrogen but is not a protein. It is probably allied to the substance that occurs in the cell membranes of the apical meristems of many roots.

8. The undulation characteristic of the Casparian strip can be produced both by swelling the cell membranes in concentrated sulphuric acid and by contracting them by heating in strong potash. It is due to the unequal expansion or contraction of the different constituents of the membrane.

9. The primary endodermis will permit the passage of water and of solutes capable of diffusing through the protoplasts, but the Casparian strip greatly impedes the passage both of water and solutes through the cell membranes.

10. The secondary endodermis is characterised by the presence of a suberin lamella, which always lines the inner tangential wall

from the inner margin of the Casparian strip, and is usually deposited all over the inner surface of the cell.

11. This suberin lamella is impregnated by substances, similar to the derivatives of suberogenic acids found in the suberin lamella of normal periderm, but on saponification no salts of phellonic acid can be identified.

12. The impregnating substances do not give the same staining reactions as those of the Casparian strip.

13. Evidence is advanced for the presence of a basal substance of unknown composition in the suberin lamella, but differing from that occasionally present in the Casparian strip in its solubility in strong sulphuric acid. The simplest tentative assumption is that this substance is a carbohydrate, a more resistant cellulose derivative, allied to the modified cellulose-like substance deposited in the internal layers of the tertiary stage endodermis in *Potamogeton*.

14. The suberin lamella renders the secondary endodermis relatively very impermeable both to water and solutes, except when passage cells are present. Experimental evidence for this conclusion is given.

15. The conclusions reached in this paper are in harmony with the views expressed in earlier papers as to the part played by the endodermis in the development of exudation pressures.

16. Some of the consequences of applying these considerations to problems of physiological anatomy are indicated by a brief discussion of the physiological relation of stelar strands to ground tissues in the Filicineæ.

REFERENCES

- (1) BÄSECKE, PAUL. Beiträge zur Kenntnis der physiologischen Scheiden der Achsen und Wedel der Filicinen. *Bot. Zeit.* **66**, p. 25. 1908.
- (2) BECKLEY, V. A. The Formation of Humus. *Journ. Agric. Sci.* **11**, pp. 66–77. 1921.
- (3) BOWER, F. O. Presidential Address to Royal Society of Edinburgh, 1920. *Proc.* **41**, p. 1. 1921.
- (4) CONRAD, M. and GUTZHEIT. Über die Entstehung und Zusammensetzung der Huminsubstanzen. *Ber. der Deutsch. Chem. Ges.* **19**, pp. 2844–2850. 1886.
- (5) CRANNER, B. HANSTEEN. Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III, Beiträge zur Biochemie und Physiologie der Zellwand lebender Zellen. *Jahrb. für Wissensch. Bot.* **53**, pp. 536–598. 1914.
- (6) ———. Beiträge zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. *Ber. der Deutsch. Bot. Ges.* **37**, pp. 380–391. 1919.

- (7) CZAPEK, F. Zum Nachweis von Lipoiden in Pflanzenzellen. *Ber. der Deutsch. Bot. Ges.* **37**, pp. 207-216. 1919.
- (8) DEVAUX, H. Recherches sur les Lenticelles. *Ann. des Sci. Nat.* VIII^e Sér. XII, pp. 1-240. 1900.
- (9) GILSON, EUGÈNE. La Suberine et les Cellules du Liège. *La Cellule*, **6**, p. 63. 1890.
- (10) HABERLANDT, G. *Physiological Plant Anatomy*. London, 1914.
- (11) HÖHNEL, F. VON. Über den Kork und verkorkte Gewebe überhaupt. *Sitzungsber. der Math.-Naturwissensch. Classe der Kais. Akad. der Wissensch. Wien*, **76**, pp. 507-660. 1877.
- (12) HOPPE-SEYLER, F. Über Huminsubstanzen, ihre Entstehung und ihre Eigenschaften. *Zeitschr. für Physiol. Chem.* **13**, pp. 66-121. 1889.
- (13) KROEMER, K. Wurzelhaut, Hypodermis und Endodermis der Angiospermenwurzel. *Bibl. Bot.* **59**, p. 1. 1903.
- (14) DE LAVISON, JEAN DE RUFZ. Du mode de pénétration de quelques sels dans la plante vivante. Rôle de l'endoderme. *Rev. Gén. de Bot.* **22**, p. 225. 1910.
- (15) MAGER, HANS. Beiträge zur Anatomie der physiologischen Scheiden der Pteridophyten. *Bibl. Bot.* **14**, Heft 66. 1907.
- (16) MANGIN, M. L. Recherches anatomiques sur la distribution des Composés pectiques. Paris, 1893. (Extrait du *Journal de Botanique*.)
- (17) MANN, ANNETTE G. Observations on the Interruption of the Endodermis, etc. *Proc. Roy. Soc. Edinburgh*, **41**, pp. 50-59. 1921.
- (18) MYLIUS, GEORG. Das Polyderm. *Bibl. Bot.* **18**, Heft 79. 1913.
- (19) OSTERHOUT, W. J. V. Some Quantitative Researches on the Permeability of Plant Cells. *The Plant World*, **16**, pp. 129-144. 1913.
- (20) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytologist*, **19**, pp. 189-200. 1920, and **21**, pp. 41-47. 1922.
- (21) — Suberin and Cutin. *New Phytologist*, **20**, pp. 17-29. 1921.
- (22) PRIESTLEY, J. H. and ARMSTEAD, DOROTHY. The Physiological Relation of the surrounding tissue to the Xylem and its contents. No. 2 in this series of Papers. *New Phytologist*, **21**, pp. 62-80. 1922.
- (23) PRIESTLEY, J. H. and TUPPER-CAREY, R. M. The Water Relation of the Plant Growing Point. No. 4—to be published in this series of papers.
- (24) PRIESTLEY, J. H. and WOFFENDEN, LETTICE M. Causal Factors in Cork Formation. No. 5—to be published in this series of papers.
- (25) RIMBACH, A. Über die Ursache der Zellhautwellung in der Endodermis der Zellen. *Ber. der Deutsch. Bot. Ges.* **11**, p. 94. 1893.
- (26) RUMPF, GEORG. Rhizodermis, Hypodermis und Endodermis der Farnwurzel. *Bibl. Bot.* **13**, Heft 62, pp. 1-45. 1904.
- (27) SCHWENDENER, S. Die Schutzscheiden und ihre Verstärkungen. *Abhand. der Königl. Akad. der Wissensch. Berlin*, **45**, p. 5. 1882.
- (28) STRASBURGER. Über den Bau und das Wachstum der Zellhaute. Jena, 1882.
- (29) TISON, ADRIEN. Recherches sur la chute des Feuilles chez les Dicotylédones. *Mém. Soc. Linn. Normandie*, XX. Sér. 2^e, 4, p. 121. 1900-1901.
- (30) VRIES, DE. Studien over Zuigwortels. *Maandbl. vor Natuurwetensch.* Bd. **13**, pp. 53-86. 1886. Abstract in *Bot. Zeit.* p. 788. 1886.
- (31) WALTER, GEORG. Über die braunwandigen, sklerotischen Gewebeelemente der Farne. *Bibl. Bot.* **4**, pp. 1-21. 1890.
- (32) WISSELIINGH, VAN. La Gaine du Cylindre Central dans la Racine des Phanérogames. *Arch. Néerl. des Sc. Exactes et Nat.* **20**, p. 95. 1886.
- (33) — Sur la Lamella subéreuse et la subérine. *Arch. Néerlandaises des sciences exactes et naturelles*, **26**, p. 305. 1892.
- (34) ZEMPLEN, GEZA. *Zeit. physiol. Chem.* **85**, pp. 173-179. 1913.
- (35) ZIMMERMANN, A. *Botanical Microtechnique*. London, 1896.
- (36) ZIEGENSPECK, H. Über die Rolle des Casparyschen Streifens der Endodermis und analoge Bildungen. *Ber. der Deutsch. Bot. Ges.* **39**, pp. 302-310. 1921.

PERMEABILITY

By WALTER STILES

CHAPTER VIII

THE PLASMA-MEMBRANE

THE presence of a layer of protoplasmic material limiting the general body of the cytoplasm and differing from it in permeability properties, is regarded by many botanists as essential for explaining the relation of the cell to water and its differential behaviour towards dissolved substances in regard to their absorption. Such membranes are, however, not morphologically obvious in most cells, and some investigators dealing principally with animal cells, have denied the existence of such protoplasmic membranes altogether. An enquiry is obviously necessary here into the evidence for the existence of such limiting membranes.

The question has not been simplified by a somewhat confused terminology. By the term plasmatic membrane or plasma-membrane Pfeffer (1877, 1890) clearly meant to denote a surface layer of the protoplasm with semi-permeable properties, but the character of which was not further defined. De Vries (1884 *a*, 1885) used the terms ectoplast and tonoplast for layers limiting the protoplasm externally in contact with the cell wall and internally in contact with the vacuole respectively. These terms were used by de Vries and later by Livingston (1903) so as to imply apparently the same as Pfeffer's term plasmatic membrane.

Again, in some cells, as for example in the plasmodia of Myxomycetes, there is a peripheral layer of the protoplast morphologically distinct from the internal mass of the protoplasm, in that under the microscope it appears clear and not granular. It is not to be assumed that this outer layer, the hyaloplasm, is identical with the plasma-membrane of Pfeffer. "A hyaloplasmic border of measurable thickness can hardly be identical with the plasmatic membrane throughout its whole extent, although the mere existence of such a border, since it indicates that its internal surface has the power of repelling granules, shows that a certain similarity must exist between its characters and those of a plasmatic membrane" (Pfeffer, 1900). The ectosarc of *Amæba* and the ectoplasm of some plant and animal cells appear to be similar to the hyaloplasm.

For the sake of clearness therefore, the term plasma-membrane will be used to denote a surface layer of protoplasm which behaves as a membrane surrounding the bulk of the protoplasm, and which may exhibit different degrees of permeability to different substances, readily permitting the passage of some, less readily permeable to others, and to yet other substances quite impermeable. The membrane bounding the outside of the protoplast, and so in contact with the cell wall, will be called the external plasma-membrane, and that bounding the vacuole, the internal plasma-membrane. For the visibly distinct outer layer of the protoplasm the term hyaloplasm will be used. Of the existence of this in certain cells there is no doubt. What we are immediately concerned with here is the existence of the plasma-membrane. Should the evidence for the existence of the plasma-membrane be regarded as convincing, the question then arises of the identity of the plasma-membrane with the hyaloplasm.

There are five main lines of evidence for the existence of plasma-membranes. These will now be considered.

I. EVIDENCE FROM THE PHYSICAL LAWS OF SURFACES

Surface phenomena have been discussed in some detail in Chapter III, where it has been pointed out that the surface of separation between two phases, such as we have at the boundary between the protoplast and the cell wall, and between the protoplast and the vacuole, has physical properties different from those of the bulk of either phase. It has been noted that one result of these different physical properties is that any substance present in solution which lowers the surface tension of the solvent will tend to accumulate at the surface. Hence substances present in the protoplasm which lower the surface tension of water will be present in the surface layer in higher concentration than in the bulk of the protoplasm. That the protoplasm contains many such substances there can be no doubt, and the presence of these substances in greater concentration in the surface layer than in the internal mass of the cytoplasm, might in itself account for a difference in permeability properties between the surface and the interior of the cytoplasm. But the cell wall and the vacuole no doubt also contain substances which lower the surface tension of water, and these will also tend to accumulate at the surface of the cell wall and vacuole respectively. Thus at the interfaces, cell wall-protoplast and protoplast-vacuole, there must be present thin layers of material which differ both physically and

chemically from the phases which they separate. These layers are no doubt largely composed of loosely bound adsorption compounds, but it is always possible that the meeting of substances adsorbed from the one and the other of the two phases in contact may result in the formation of more stable chemical compounds which are not present in the interior of either of the two adjoining phases. In such a case the plasma-membrane would be regarded as produced in much the same way as the precipitation membranes discussed in Chapter V. The formation of a rigid film at the surface of separation of a protein solution and some other phase (Ramsden, 1903), presumably by the protein adsorbed at the surface undergoing some irreversible change, is of significance in this connection.

In any case it is clear that from purely physical and physico-chemical considerations there is good reason to suppose that where the protoplast is in contact with the cell wall and the vacuole there are thin layers which differ in physical and chemical properties from the general mass of the protoplasm. It is possible and, indeed, to be expected, that among other properties in which these layers differ from the internal cytoplasm, is their permeability to different substances.

2. EVIDENCE FROM CYTOLOGICAL OBSERVATIONS

It is convenient to group under this second heading all the evidence bearing on the existence of plasma-membranes which is based on actual observations made on the protoplasm of the cell. Such observations are of three kinds: (1) ordinary microscopic observations, (2) ultramicroscopic observations, and (3) those made by the aid of microdissection.

(1) *Microscopic observations.* Incidental reference has already been made to the clear hyaloplasmic layer of Myxomycetes and of the cells of other organisms. The hyaloplasm is presumably more viscous than the internal mass of the cytoplasm as it appears free from moving granules which would thus seem to be repelled from entering it. It is to be noted that the existence of the hyaloplasm appears to be due to surface forces, for it remains constant in thickness when the cell increases or diminishes in volume owing to absorption or loss of water (Bayliss, 1915).

Sometimes in cells that have been killed a very fine membrane can be recognised limiting the now dead protoplasm. As Bayliss (1915) points out, this "is no proof that the membrane was in existence in the living cell in the same state as that seen."

The method of de Vries (1885) of rendering visible the inner plasmatic membrane, by plasmolysing with 10 per cent. potassium nitrate and staining with eosin, is open to similar objection.

In experiments on the penetration of a number of dyes into intact cells of a number of plant and animal species, and through the internal protoplasm of torn cells, Kite (1913 *a*, 1913 *b*, 1915) finds the surface layer of the cells he investigated is generally more easily penetrated by a dye than the interior protoplasm. So far, however, from regarding this as any evidence of the existence of a limiting plasmatic membrane, he speaks of such membranes as hypothetical structures. Although his results are in direct contradiction to the general opinion of the permeability properties of plasmatic membranes, it is not clear how he comes to regard such membranes as non-existent.

(2) *Ultramicroscopic observations.* The observations of Gaidukov and Price with dark-ground illumination have been referred to in Chapter II. Some of their observations relate to the plasma-membrane.

Gaidukov (1910) records a differentiation between the outer layer of the cytoplasm and the internal part of the cell, but it is not clear from his description whether this outer layer can be referred to hyaloplasm or not. The observations of Price (1914) are more definite. In the cells of the leaf of *Elodea canadensis* "no definite differentiation into endoplasm and ectoplasm was made out." In the cells of the large multicellular hairs of the stems and leaves of *Cucurbita* spp. "there is what appears to be a slight membrane limiting the protoplast towards the vacuole, and also forming the surfaces of the strands" (that is, of protoplasm). In the cells of the hairs of the tomato an outer layer of the cytoplasm appears to be differentiated from the rest of the cytoplasm, the particles in Brownian movement being smaller and less numerous than in the main mass of the protoplasm. In a series of observations made on the germination of spores of a species of *Mucor*, the contents of the ungerminated spore appeared almost homogeneous, but after absorption of water had taken place for several hours a peripheral layer with the appearance usually attributed to a gel became differentiated from the general mass.

Price also studied the appearance of plasmolysed cells under dark ground illumination. He states that in *Spirogyra* plasmolysed with dilute glycerol a layering of the protoplast can be observed, an external layer containing extremely fine particles becoming differentiated from the inner region containing the larger microsomes.

The outer layer appears to be drawn out in places into threads which at their outer end retain contact with the cell wall. Such fibrils have frequently been recorded as occurring in plasmolysis (Gardiner, 1884; Bower, 1885; Chodat and Bourbier, 1898; Hecht, 1912). The particles in the outer region were in active movement, and there was no sign of a hydrogel layer limiting the protoplast, but Price suggests that some definite boundary must be present as the colloid particles do not escape into the surrounding liquid. Cells plasmolysed with 10 per cent. potassium nitrate presented a similar appearance.

Sometimes during the plasmolysis of *Spirogyra*, vesicles of protoplasm containing presumably cell sap are formed (cf. de Vries, 1885). The wall of the vesicle contains particles in active movement and if any part of the protoplasm is in the gel state it must be an extremely thin layer, for none is obvious. In *Mougeotia* on the contrary, the wall of the vesicle appears to be rather in the gel condition.

During plasmolysis of the cells of the hairs of *Cucurbita* the protoplast appears to be bordered on the outside by a definite transparent membrane on which finer particles in the interior of the protoplast appear to impinge. This membrane was observed when plasmolysis was brought about by 30 per cent. sucrose, and so is presumably to be regarded as normal and not produced by the coagulating action of electrolytes.

It is scarcely possible to draw any very definite conclusion from this work with regard to the general presence of a plasma-membrane. In the hairs of the tomato the outer layer differentiated from the rest of the cell contents appears to be identical with hyaloplasm; in the hairs of *Cucurbita* there appears to be a thinner layer of a gel nature, limiting the protoplast on the outside.

(3) *Evidence from the results of microdissection.* The observations made on the structure of protoplasm by means of microdissection have already been mentioned in Chapter II. The conclusions to which workers with this method come in regard to the plasma-membrane will now be indicated.

Chambers (1917) describes the peripheral layer of the cytoplasm of young germ cells, egg cells and Protozoa as much denser than the interior cytoplasmic sol, but merging insensibly into it, and concludes that this surface layer is a highly extensile, contractile and viscous gel. If this surface layer is injured a new and similar layer will form at the damaged place, the capacity for the establishment and maintenance of the viscous gel layer being a property essential to cytoplasm. The rate of diffusion of three dyes, neutral red, cresyl blue and janus

green, appeared to be the same in the surface layer as in the general body of the cytoplasm. In adult somatic cells, where the general mass of the cytoplasm is in the gel state, it is not possible to distinguish a differentiated outer layer.

The peripheral layer of gel observed by Chambers in young germ cells, egg cells and Protozoa, appears to be the hyaloplasmic border, and in the case of the Protozoa it is definitely spoken of as the ectoplast. Seifriz (1921) has, however, recently made a special study of protoplasmic membranes by the microdissection method, and concludes that in *Amœba* and the plasmodium of Myxomycetes the hyaloplasmic border is not to be regarded as the plasma-membrane, for there is evidence of a definite, though exceedingly delicate membrane limiting the hyaloplasm on its outer side. It is shown that the surface of a young and active pseudopodium is liquid, for if the hyaloplasm is pierced by a microdissection needle and this moved towards the edge of the protoplasm, the hyaloplasm will follow the needle so as to form an artificial pseudopodium, while on releasing the needle there is no appreciable contraction. This behaviour is only explicable on the view that the surface layer is liquid. But after the pseudopodium has advanced to a certain point, there is no further advance, to explain which it is supposed that a sudden change in consistency of the protoplasm from sol to gel takes place. This change must be at the surface, for the hyaloplasm changes little in viscosity. The surface layer of gel is now capable of a little stretching. This is the condition of a quiescent plasmodium. Seifriz thus concludes that "the inactive surface layer is a highly viscous emulsion colloid, undoubtedly in the gel state, which solates (*i.e.* becomes a sol) when streaming takes place, and reverts to the gel state when the plasmodium again becomes inactive. This surface layer is exceedingly delicate, of immensurable thickness, and is not identical with the hyaloplasmic border (or ectoplast), which, to be sure, it resembles in constitution, but from which it is more or less sharply delimited." The state of affairs in *Amœba* is similar except that the hyaloplasm (ectoplast) behaves in the same way as the membrane, though in the passive condition it is less rigid than the membrane and so can be distinguished from it.

From the fact of the regeneration of the plasma-membrane from cytoplasm containing hyaloplasm, it is concluded that the membrane is formed from the latter and is certainly not to be regarded as an autonomous organ as de Vries (1885) supposed.

In dead material a delicate membrane can sometimes be observed

surrounding the protoplasm. This is regarded by Seifriz as the plasma-membrane after it has become changed by "fixing" or coagulation. The occurrence of this "degenerate" membrane is cited by Seifriz as further evidence for the existence of the plasma-membrane. There is similar evidence for the existence of an internal plasma-membrane surrounding the vacuole, and also one separating the protoplasm and nucleus (the nuclear membrane).

It is to be noted particularly that the plasma-membrane is capable of undergoing reversible change from sol to gel, of readily adjusting itself to changes of shape and area, and if destroyed is in most cases readily and immediately re-formed at the surface of the cytoplasm, from which it is not capable of isolation until the cytoplasm has undergone degeneration. It is obviously to be regarded as part of the protoplasm.

3. EVIDENCE DERIVED FROM A CONSIDERATION OF THE WATER RELATIONS OF THE CELL

The simplest case that can be considered in this connection is that of a cell unprovided with a cell wall and in which vacuoles are not present. Such cells, common in animals, are comparatively unusual in plants. Animal cells are often surrounded by a solution of a number of salts, and although the concentration of this solution varies in different cases, the salts present in it are very generally those of sea water, and the relative proportion of the salts may be the same as in that medium. If cells, for example, red blood corpuscles, or pieces of tissue such as muscle or sheep's eyes, are placed in distilled or tap water, the cells absorb water, and this absorption may continue until the swelling is such that the cell or tissue is ruptured. The cells are thus behaving as if they consisted of a solution possessing an osmotic pressure and surrounded by a semi-permeable membrane. If this be the case, as the osmotic pressure exerted by the colloids of the cell must be very small, while the movement of water into the cell indicates a very considerable osmotic pressure, the existence of the latter must be due to crystalloids.

This is the usually accepted explanation of the swelling of cells in distilled water or in weak solutions, and of the corresponding shrinkage of cells in solutions of high osmotic pressure. It involves the assumption of a semi-permeable membrane enclosing the general body of the protoplasm. As Loeb (1906) has truly pointed out, it is not necessary that the membrane should be impermeable to salts for the absorption of water from a solution of such salts to take place,

for if the crystalloids only diffuse slowly into the protoplasm, while the membrane offers very little opposition to the passage of water, absorption of water will take place readily at first.

In young plant cells vacuoles are either non-existent or very small, and the system thus approaches very closely to such a cell as that considered above, but surrounded by a wall. The behaviour of such a cell towards water is similar to that already described, but the presence of the wall is responsible for certain differences. Thus, when the walled cell is immersed in water or in a dilute solution, water is absorbed, but this cannot proceed as rapidly or to the same extent as in the case of the unwalled cell, for as water passes into the cell the increase in volume involves a stretching of the cell wall which thus exerts a continually increasing tension inwards, so that on account of this water tends to be forced out from the cell. When these two forces, that forcing water in and that forcing it out, equal one another, an equilibrium condition is reached, and the protoplast is thus prevented from absorbing water until it bursts. With thin cell walls this can however sometimes happen. Thus some marine algæ, notably *Derbesia* and *Bryopsis*, when transferred to fresh water (Noll, 1888), will burst very easily. Lidforss (1896) has recorded the bursting of pollen grains of a number of species, notably those belonging to the Liliaceæ, in the same way. F. E. Lloyd (1915, 1916, 1917 *a*, 1917 *b*) has observed the bursting of pollen tubes of *Lupinus*, *Lathyrus* and *Phaseolus* in distilled water or weak sucrose solutions. The same author has even observed the bursting of pollen grains of *Gossypium* in 50 per cent. glycerol, 25 per cent. sucrose and 0.45 *N* potassium nitrate, surprisingly high concentrations. The tips of the hyphæ of moulds (Curtis, 1900) and the asci in some Ascomycetes (Livingston, 1903) will burst if placed in distilled water or in a very weak solution.

The water relations of the non-vacuolated cell can thus be easily explained if it is assumed that the outer layer of the protoplast consists of a membrane which is readily permeable to water but impermeable, or only comparatively slightly permeable, to many soluble substances present in the protoplasm. Such a view, as has already been stated, has its opponents, one of the most ingenious of whom, M. H. Fischer, has attempted to explain the absorption of water by organic tissue on account of the colloidal nature of the tissue. In a long series of experiments Fischer (1910) has shown that the swelling of certain proteins, namely gelatine and blood fibrin, in water and in solutions of acids, salts and alkalies, closely resembles

the swelling of tissues as exemplified by frog's muscle and sheep's eyes.

Miss D. J. Lloyd (1916) has also made a study of the swelling of excised sterno-cutaneous muscle of the frog in solutions of acids, alkalies and salts. She comes to the conclusion that the swelling of muscle both inside and outside the body is an osmotic phenomenon, and compares her results with those of Lillie (1907), who showed that acids and alkalies bring about an increase in the osmotic pressure of gelatine and egg-albumin. Miss Lloyd accepts the view that acids combine with colloids to give ionisable complexes which dissociate into a large "pseudo-ion" carrying a positive charge and a mobile ion with a negative charge; the degree of dispersity of the system is increased and so the osmotic pressure is raised (cf. Hardy, 1900). Similar considerations hold with regard to the action of alkalies. Miss Lloyd thus agrees with Procter (1914) in ascribing to ionisation the production of the internal osmotic pressure of such colloids, but differs from him in supposing that the colloidal ion is partly responsible for the osmotic pressure and not merely the mobile ion. In solutions of neutral salts and in sugar solutions (for the latter see Hardy, 1905), muscle may show a preliminary gain in weight, but sooner or later coagulation sets in and loss in weight supervenes (cf. Lillie's results with gelatine, 1907). Miss Lloyd ascribes this to the suppression of the ionisation of the colloid particles of the muscle, which lose their charge and are coagulated.

Miss Lloyd holds that there is no need to postulate the presence of a semi-permeable membrane surrounding the muscle fibres, the structure of the colloid in itself being presumably such as to prevent the movement of the colloidal ions and consequently also of the oppositely charged mobile ions.

Loeb (1916) has suggested a causal link between the development of acidity in living muscle cells and the imbibition of water. It is suggested that the acid brings about a hydration of the proteins to such an extent that the true solutes are now held in a very reduced volume of water. This marked rise in osmotic pressure brings about the entrance of water into the cell.

The observations of F. E. Lloyd (1915, 1916, 1917 *a*, 1917 *b*) on the effect of acids and alkalies on the growth of pollen tubes are of particular interest in the present connection. Lloyd grew pollen tubes in hanging drops of a solution of sucrose to which had been added a definite quantity of acid or alkali and made observations on the rate of growth of the tubes. In pure sucrose solutions the rate

of growth of the tubes was found to be inversely proportional to the concentration of sucrose, but if the concentration of the sugar was too low, the pollen tubes burst (see above). Thus the greatest rate of continued growth occurred in a solution of about 20 per cent. sugar. Since growth depends on the swelling of the protoplasm, that is, on the capacity of the protoplast to absorb water from the external medium, the introduction of substances into the external medium which alter the imbibition capacity of the protoplast differently from the osmotic pressure should give a means of determining whether the absorption of water by the cell is due to imbibition by the protoplast on account of its colloidal nature, or on account of differences in osmotic pressure on two sides of a semi-permeable membrane.

Lloyd found that acids (hydrochloric, nitric, acetic and citric) added to 20 per cent. sucrose, did not affect the growth rate when the concentration of acid was between $N/51200$ and $N/3200$ but that in higher concentrations, from $N/1600$ to $N/200$ the growth rates were less and less with progressive increase in the concentration and always terminated by bursting. When, however, acetic acid was added to 40 per cent. sucrose the growth rate when the acid was present in a concentration of $N/3200$ was increased to about four times that of the control. With alkalis added to 20 per cent. sucrose growth was depressed when the alkali was present in a concentration of $N/400$, but was greater when the concentration of alkali was within the range $N/800$ to $N/12800$, the greatest rates being with alkali concentrations of about $N/1600$ and $N/3200$. At the higher concentrations bursting of the tubes took place.

It will be noticed that the concentrations of acid and alkali used are negligible in comparison with that of sugar employed and so will not exert any appreciable influence on the value of the osmotic pressure of the external solution. On the other hand, the observations of many observers (for example, Pascheles, 1897; Wo. Ostwald, 1905, 1906; M. H. Fischer, 1910; Procter, 1911, 1914; Loeb, 1918) have shown that the swelling of gelatine and other colloids is greatly influenced by the hydrogen-ion concentration of the external medium. Lloyd has himself made experiments on the influence of acidity and alkalinity on the rate of swelling of gelatine. He concludes that for all acids there is a concentration which induces a maximum rate of swelling and a concentration which depresses swelling to a rate less than that of pure water. Alkalies produce a similar effect but it is not yet certain whether the minimum swelling rates are less than those occurring with distilled water.

From these experiments Lloyd thus comes to the conclusion that imbibition by the protoplasm rather than osmotic pressure is the dominant factor in growth.

While in general the water relations of the non-vacuolated cell can be explained by assuming the presence of a semi-permeable layer limiting the protoplast, there is yet a considerable body of evidence and opinion indicating the importance of imbibition by the colloidal protoplasm in determining the uptake and excretion of water by the living cell. Yet it has been shown that change in volume of cells of the kidney (Siebeck, 1912) and muscle cells (Beutner, 1912 *a*) placed in solutions of a substance which does not enter the cell are those which are to be expected if the protoplast is surrounded by a semi-permeable membrane. If the loss or gain in water by the cells were related solely to the imbibition properties of the protoplasm, a quite different relation should hold.

The evidence in regard to the presence of a semi-permeable membrane limiting the protoplasm of the non-vacuolated cell is thus rather conflicting. The observations at present available are much too scanty for us to draw any definite conclusions. There seems, however, to be definite evidence that in some cases at any rate, imbibition by the protoplasm plays a very definite part in determining the intake of water into the cell. How far the presence of a semi-permeable membrane is necessary for an understanding of water intake into the cell has still to be made clear.

Whatever may be the case with the non-vacuolated cell, there appears to be good reason for supposing that the intake of water by the vacuolated cell can be largely related to osmotic phenomena. Whether or not the outer layers of the protoplast are differentiated from the bulk of the protoplast so as to constitute semi-permeable membranes differing in permeability from the rest of the protoplast, it is reasonable to suppose that the essentially colloidal protoplast must itself act as a semi-permeable membrane between a liquid external to the cell and that contained in the vacuole.

4. EVIDENCE FROM THE FACTS OF SELECTIVE PERMEABILITY AND ALLIED PHENOMENA

A great number of observations have been made which indicate that different substances penetrate into cells at very different rates and to very different extents. This behaviour of the cell is directly comparable to the behaviour of the membranes considered in Chapter V,

and so it is very natural to account for this differential intake of substances into the cell by the presence of a superficial layer of the protoplasm which acts in the same way as an artificially prepared precipitation membrane. Other phenomena, such as the presence in the protoplasm of dissolved substances, including free electrolytes (Höber, 1912 *b*, 1913), which do not diffuse out into the external medium, and the deplasmolysis of plasmolysed cells in glycerol and various salt solutions, are also readily explained on the same assumption. Also artificial membranes of collodion impregnated with calcium phosphate (Meigs, 1913) or an ethereal extract of muscle (Philippson, 1913) have been prepared which behave very similarly to certain cells in regard to the passage of salts.

We have already seen when considering the evidence for the presence of a plasma-membrane yielded by the facts of the water relations of the cell, that there are those who deny the existence of such semi-permeable membranes. In the present connection reference may be made more particularly to the opinions of Moore and Roaf (1908), who state that the membrane theory fails to explain (*a*) the difference in composition of electrolytes in the corpuscles of blood and in the serum surrounding them; for instance, the concentration of chloride in the serum was found to be about three times the concentration of this ion in the corpuscles (and see also Abderhalden, 1898 and Höber, 1912 *a*, for further data); (*b*) the physiological effects of perfusion by media defective or excessive in certain electrolytes and normally present in the cell; and (*c*) the selective uptake of certain ions. Moore and Roaf hold that these effects are simply explained on the view that the cell proteins or other substances of the protoplasm form chemical or adsorption compounds with ions. The cell, when functioning normally, is held to be in a state of mobile equilibrium so that it can undergo reversible changes involving these electrolytic constituents, and presumably others, whereby they become associated with the cell protoplasm or the reverse. These writers thus suggest that selective uptake is to be explained, not by the selective permeability of a membrane, but by specific affinities of the cell protoplasm for certain ions, whereby they are adsorbed or combine chemically with the protoplasm, while the quantitative relation between the concentration of an electrolyte or ion in the cell and in the environment is maintained at a definite equilibrium which depends on the adsorption equation or molecular affinities, and which will therefore not as a rule correspond to equality of concentration inside and out. Similarly it is pointed out by Moore,

Roaf and Webster (1912) that if a cell is washed with a solution either free from, or very poor in, a particular ion, the amount of this ion washed out from the cell may be practically inappreciable, for it will only be washed out until the concentration of the ion in the external solution bears to the concentration of the ion in the cell the relation required by the adsorption equation or molecular affinities, and under these circumstances the equilibrium concentration of the ion in the external solution may be very low indeed.

In supporting the membrane theory, Bayliss (1915) deals particularly with the view that the apparent impermeability of cells to some salts can be sufficiently accounted for by the presence of a membrane semi-permeable as regards colloids, but permeable to crystalloids. This view does not differ greatly from that of Moore and his collaborators already considered. To such a view Bayliss raises the following objections. Firstly, as, if a membrane is permeable to one ion of an electrolytically dissociated salt and not to the other, it is impossible for the salt or either ion to pass through the membrane (see Chapter V), if we have a salt of a protein which is electrolytically (but not hydrolytically) dissociated, and the protein ion is incapable of passing through the membrane, the other ion of the compound will also be incapable of doing so. But if there are two colloidal salts present, in one of which the kation is diffusible and in the other the anion, as there must presumably be if a neutral salt is taken into the cell and both ions so held, then both ions will diffuse readily out from the cell, so that in such a case a hypothesis not involving a membrane exhibiting complete or partial semi-permeability to such salts will not explain the observed facts. Secondly, Bayliss thinks there is no satisfactory evidence for the presence of compounds between proteins and neutral salts. Thirdly, glucose does not form a compound with proteins of the form required by the hypothesis, but exists free in the blood according to Asher (1912), and is apparently indiffusible into the corpuscles under normal conditions. Fourthly, the high osmotic pressure of certain cells cannot be given by substances of such high molecular weight as proteins, and seems only accountable by the presence of substances of comparatively low molecular weight in an osmotic cell. Finally, difficulties arise with regard to the distribution of different ions within and without the cell if a membrane more or less impermeable to crystalloids is dispensed with. It has been shown both by Bayliss (1911) and Donnan (1911) that it is possible to have different concentrations of a salt on the two sides of a membrane through which the salt can diffuse freely, but that **in**

this case the facts of distribution differ very considerably from those in the case of blood serum and corpuscles, for example. Thus, if, for instance, the sodium salt of a protein is separated from pure water by a membrane of parchment paper, the diffusible sodium ions can only diffuse to a position in which their osmotic pressure is balanced by the electrostatic attraction of the non-diffusible oppositely charged protein ion. Now if the pure water of the external medium is replaced by a solution of a freely diffusible salt, as for example potassium chloride, the potassium ions and chlorine ions can both diffuse through the membrane, and some of the potassium ions can replace the sodium ions in relation to the negatively charged protein ions, and this process will proceed until the sodium and potassium ions are distributed throughout both solutions in the same relative proportions. But the absolute concentrations of the two kations will be different on the two sides of the membrane. Now in the case of rabbit blood (Abderhalden, 1898) the potassium bears a different ratio to sodium in the plasma from that which it bears in the corpuscles, so that the presence of a colloidal salt within a membrane permeable to crystalloids does not account for the distribution of ions in this particular case.

In reviewing the evidence given by a consideration of the relation of solutes to the cell in favour of, or against, the presence of a semi-permeable plasma-membrane, it is again necessary to distinguish the cases of the vacuolated and non-vacuolated cell. Failure to do this in the past has undoubtedly led to the drawing of conclusions improperly. It has already been indicated that the protoplasm of the vacuolated cell fulfils the conditions necessary for a membrane between the vacuole and the external medium, and one which may exhibit differential permeability to different substances. The retention of sugar and the red pigment by the cells in slices of root of red beet can be explained satisfactorily on these grounds. Similarly the deplasmolysis of vacuolated cells such as those of *Spirogyra* and of the staminal hairs and epidermis of *Tradescantia* when plasmolysed in glycerol or solutions of certain salts is to be explained by the gradual passage of the solute through the protoplasm into the vacuole. In order to obtain evidence in relation to the presence of a plasma-membrane we shall do well to devote special attention to data derived from a study of the relation of solutes to non-vacuolated cells. Although Pfeffer (1900) admitted that no absolutely convincing proof had been brought forward, he gave reasons for supposing that the limiting membranes have different osmotic properties from the

rest of the protoplast (Pfeffer, 1890, 1900). Thus, when a cell is treated with very dilute hydrochloric acid, the plasmatic membrane assumes "a condition of rigor," but its original diosmotic properties are usually at first retained, and a dye, which is unable to penetrate the plasma-membrane will rapidly penetrate throughout the dead protoplast if it can find entrance through a tear or gap in the membrane. The fact, already referred to, that the vacuolar membrane may actually be separated by strong and sudden plasmolysis, is cited as a further argument. The reader must form his own judgement of the value of these lines of evidence derived from pathological conditions; to the present writer they are very unconvincing, and it is to be noted that Pfeffer himself did not press them.

With regard to non-vacuolate cells, then, it must be admitted that the evidence is insufficient to enable one to come to any conclusive decision as to the presence of a limiting protoplasmic membrane. But here again, as with the water relations of the cell, it is impossible to avoid the conclusion that substances may be able to pass into the protoplasm and may be held there simply on account of the colloidal properties of the protoplasm. The cases dealt with by Bayliss are much simpler than those presented by living cells. When the state of equilibrium between the very complex system which constitutes the latter, and the external medium, is disturbed by changes in the composition of the external medium, it is clear that there may be expected far-reaching changes in regard to molecular association in the protoplasm, changes which may involve adsorption of substances from the outer liquid, chemical combination, or breaking down of existing molecular associations in the protoplasm to bring about a new condition of equilibrium. While the possibility of a limiting semi-permeable plasma-membrane is not ruled out, the evidence at present available from a consideration of the relation of the cell to substances dissolved in the medium outside it, is by no means overwhelmingly in favour of the presence of such a membrane.

An observation made by Osterhout (1913 *b*) on the marine alga *Griffithsia* may be taken as an example of one line of evidence adduced in support of the different permeabilities of the two surfaces of the protoplasm. If cells of this alga are placed in a solution having the same relative composition as sea-water, but stronger, plasmolysis takes place, and on returning the cells to normal sea-water the protoplasts regain their original size. But if instead of sea-water a solution of ammonium chloride is used as plasmolysing solution,

contraction of the protoplast from the cell wall also takes place, but the vacuole contracts much more so that the width of the protoplast may increase greatly at the expense of the vacuole.

Osterhout offers two possible explanations of this observation. Firstly, the outer surface of the protoplasm may be more permeable to ammonium chloride than the inner, or secondly, the ammonium chloride may produce an alteration in the permeability which causes a contraction called "false plasmolysis¹." If this false plasmolysis of the inner surface is greater than that of the outer, the observed effect would result.

This observation may, of course, be truly explained on the basis of semi-permeable membranes surrounding both the protoplasm and the vacuole, but it might also be explained by supposing that the ammonium chloride altered the swelling capacity of the protoplasm as a result of which it absorbed water from the vacuole with the observed result.

5. EVIDENCE DERIVED FROM A CONSIDERATION OF THE ELECTRICAL CONDUCTIVITY OF LIVING CELLS AND TISSUES

Living cells and tissues have a remarkably high electrical resistance (Stewart, 1897; M'Clendon, 1910), which rapidly falls if the cells or tissues are treated with a reagent bringing about the death of the cells (Osterhout, 1912 *b*; Stiles and Jørgensen, 1914 *a*). This phenomenon is easily explained by supposing that in the living cell the plasma-membrane offers considerable resistance to the passage of ions across it, but that when the cell is killed the organisation of the plasma-membrane is altered so that it becomes readily permeable to ions. That a visible change does often take place in the surface layer of the cell at death has already been noted.

Other explanations of these phenomena are however possible. Thus if electrolytes in the cell were held in molecular association with the cell colloids there would exist a system of high electrical resistance, while death of the cell might involve a breaking down of these compounds and the setting free of electrolytes. However, Höber (1910, 1912 *b*, 1913) has shown by ingenious electrical methods that free electrolytes are present in the cells of red blood corpuscles.

¹ Such a contraction has been noted by Osterhout (1908 *a*, 1913 *c*) when cells are immersed in dilute solutions of various salts or even in distilled water. This evidently has nothing to do with true plasmolysis.

SUMMARY OF THE EVIDENCE FOR THE EXISTENCE OF
LIMITING SEMI-PERMEABLE PLASMA-MEMBRANES

Physico-chemical considerations indicate that the surface layer of the protoplasm must differ physically and chemically from the rest of the latter, the actual composition of the limiting layer depending not only on the composition of the protoplasm, but also on that of the medium with which the protoplasm is in contact. Any change in such a medium will bring about changes in the limiting layer. Ultramicroscopic, as well as microscopic, observations, including those made with the aid of microdissection, show that in some cells there may be a comparatively wide border (the hyaloplasm or ectoplasm) differentiated from the internal protoplasm, but that there may also be a much more delicate limiting layer which is regarded as the true plasma-membrane. These lines of investigation do not give definite information on the question whether the permeability properties of the limiting layer differ considerably from those of the internal protoplasm, except that as the surface layer should be more concentrated and viscous, it should offer greater opposition to the passage of dissolved substances (compare Chapter IV).

The relations of the cell to water and dissolved substances are generally regarded as yielding evidence of the existence of a semi-permeable membrane bounding the protoplast, but an analysis of the evidence indicates that it would be premature to draw such a conclusion from existing data. The low electrical conductivity of tissues affords some evidence of the presence of a semi-permeable membrane. In this connection, unfortunately, experimental observations are very scanty, while with plants observations on non-vacuolate cells appear to be non-existent.

Conclusions appear to have been drawn incorrectly in the past owing to the distinction between vacuolate and non-vacuolate cells not having been drawn with sufficient definiteness. In the case of the vacuolate cell there appears to be every reason to believe that the protoplasm acts as a membrane, to some extent semi-permeable, separating the vacuole from the external medium.

COMPOSITION OF THE LIMITING LAYER

This is a question that has given rise to very considerable discussion.

We have noticed that dissolved substances which lower the surface tension of the solvent tend to accumulate in the surface layer. For this reason the various constituents of the protoplasm may be expected to be present in the plasma-membrane. Among substances

present in the cell are various fatty substances which lower the surface tension of water considerably. These fatty substances include the true fats which are glyceryl esters of higher fatty acids, the lipines (Leathes, 1910), which are complex compounds of fatty acids with nitrogen-containing groups, and phospholipines containing phosphorus in addition, of which the best known is lecithin. Although Quincke (1879, 1888, 1894) had earlier conceived the protoplast as surrounded with a film of oil, undoubtedly the popularity of the theory of a lipid plasma-membrane dates from the researches of Overton (1895, 1896, 1897, 1899 *a*, 1899 *b*, 1901) who showed in a series of investigations with a number of different cells and a large number of substances, including aniline dyes, that those substances soluble in lipoids easily enter the cell, while those which are insoluble in lipoids do not readily penetrate living cells. Meyer's theory of narcosis (1899) rests on a similar foundation.

In this connection Overton laid particular stress on the importance of lecithin and also cholesterol, which is not a true lipoid substance at all, but a complex alcohol (Windaus and Stein, 1904 *a*, *b*). Czapek (1910 *a*, 1910 *b*, 1911 *b*, 1914) on the other hand, rather emphasised the importance of the simpler neutral fats. He came to his conclusions as the result of a series of experiments on the exosmosis of tannin from plant cells, mainly those of the sub-epidermal mesophyll of the leaf of *Echeveria*, brought about by treatment with various toxic substances. Czapek came to the conclusion that, with few exceptions, for exosmosis to occur the concentration of the dissolved substance must be great enough to lower the surface tension of the solution (against air) to 0.68, that of pure water being taken as unity. He further found that a strong emulsion of neutral fat has a surface tension against air of 0.68, and that this is a minimal value; however strong the emulsion the surface tension is not lowered below this. Czapek was thus led to think of the surface layer of the cell as a fatty emulsion, which might indeed contain other substances, but of which the principal constituents are neutral fats. On surrounding the cell with a solution of lower surface tension than 0.68, a breaking down of the organisation of the semi-permeable membrane is supposed to result with consequent exosmosis of the contents.

Some substances, however, were found to produce exosmosis when presented to the tissue in solutions of considerably higher surface tension than 0.68. To such substances Czapek attributes a specific toxic action, the substances in question apparently acting chemically on the limiting membrane.

In a series of observations on the exosmosis of electrolytes from plant tissue, principally potato tuber, Stiles and Jørgensen (1917 *a*) have shown that it is impossible to find for any substance among a number examined, a critical concentration below which exosmosis will not take place. Exosmosis takes place in all concentrations examined, slowly at first, then more rapidly, and as equilibrium is approached, more slowly again. These stages are passed through more rapidly the more concentrated the solution of toxic substance. Czapek simply selected an arbitrary time and decided that if exosmosis had not taken place in that time it was not going to, with the exception that in some cases this time was arbitrarily extended; in other cases rapid exosmosis was attributed to secondary injury. Such methods of enquiry are not very convincing, and Koltzoff (1912) criticised Czapek for neglecting the time factor in his experiments. Czapek replied to this (1914) that he dealt only with equilibrium conditions and a time factor does not therefore enter into the question. But unless the progress of an action is followed with time, it is not always possible to decide whether equilibrium conditions have been reached. In those experiments of Czapek in which exosmosis had not occurred after the lapse of an arbitrary time, it is at least conceivable that prolongation of the time of action might have revealed exosmosis.

An important difficulty in Czapek's theory pointed out by Koltzoff and Vernon (1913) arises from the fact that the surface tensions of the solutions used in his experiments were measured against air, while in the experiments themselves it is the surface tension of the solution against the outer layer of protoplasm which is in question, while by no known means can the surface tension between two immiscible liquids be determined from their surface tensions against air. Hence it is not at all likely that even in Czapek's experiments those concentrations of different substances just producing exosmosis had the same surface tensions under the experimental conditions in question. The experiments of Stiles and Jørgensen have further shown that solutions which produce equal exosmosis of electrolytes have not necessarily the same surface tension against air. Having regard to this fact and to the exceptions to the rule recorded by Czapek himself, it seems clear that the permeability changes in the cell produced by toxic substances cannot be referred to surface tension alone.

Further arguments against the acceptance of Czapek's conclusions are that there appears no reason why a solution the surface tension

of which against air is less than that of protoplasm against air should bring about cytolysis, while finally Czapek himself quotes examples of fats, aqueous emulsions of which lower surface tension considerably below the critical value of 0.68.

An argument frequently advanced against the theory of a lipid limiting membrane is that water, which enters most cells so readily, could not so pass through a continuous film of fatty substance. This argument, however, can scarcely be maintained against Czapek's view that the fat does not form a continuous layer, but is in the form of an emulsion. Also lecithin, on which Overton laid stress, when shaken up with water, forms an emulsoid system consisting of a less aqueous disperse phase in a more aqueous dispersion medium, and it is presumably in this form that the lecithin would be held in the surface layer of the protoplasm. Unfortunately for Overton's theory, lecithin in this form is not a solvent for lipid soluble substances only (Nathansohn, 1904 *a*). Membranes of lecithin, and also of cholesterol, were found to be completely impermeable to all dyes examined, while when the lecithin membrane was saturated with water, dyes both soluble and insoluble in lipoids penetrated the membrane, not merely the lipid-soluble ones (Ruhland, 1909 *a*). For a further discussion of the properties of lecithin and cholesterol in regard to cell permeability reference may be made to Bayliss (1915). Ruhland (1912, 1913 *a*, 1913 *b*, 1914) moreover, now holds that the intake or non-intake of dyes and other substances by plant cells depends rather on the size of the molecules or molecular aggregates of the dyes, and not on their solubility or otherwise in lipid substances.

Pfeffer (1900) regarded the plasma-membrane as largely composed of protein, as indicated by the "fixation" of the limiting layer on treatment of the cell with various reagents such as dilute acids, mercuric chloride and iodine, and by microchemical tests applied to the membrane after fixation. The action of dilute salt solutions in rendering the cell more and more permeable (Pfeffer, 1877, 1890; de Vries, 1885) could also be explained as due to coagulation of protein in the membrane. Pfeffer realised that other substances might also be present in the limiting layer.

Robertson (1908) pointed out that Overton's theory is invalid for a variety of reasons, the most cogent of which is the fact that the relation between solubility in lipid substances and power to enter the cell does not hold generally. Robertson found no correspondence between penetrability and power to enter cells in the dyes examined

by him. Robertson is of opinion that the maintenance of the integrity of the cell surface can be adequately accounted for by supposing that the cell is surrounded by a thin concentrated film of protein. Such films are thought to exist very generally, and perhaps universally, at the bounding surfaces of droplets of protein solutions (cf. also Ramsden, 1903). Robertson thinks it possible that a discontinuous layer of lipid substance may underlie the outer film of protein.

Lepeschkin (1910 *a*, 1910 *b*, 1911 *a*, 1911 *b*), while agreeing that lipid substances may be present in the plasma-membrane, adduces experimental evidence in favour of the importance of proteins therein. The protoplasm of plant cells coagulates at definite temperatures, the protoplasm of *Spirogyra* sp. at about 50·5° C., that of the epidermal cells of *Tradescantia* sp. at about 70·7° C. This behaviour is similar to that of proteins (cf., for example, Schryver, 1913). The coagulation is visible in plasmolysed cells under the microscope owing to the change from a shining homogeneous to a granular appearance. At the same time there is a sudden increase in permeability made obvious by the sudden shrinkage of the protoplast. Coagulation of the protoplast by organic substances can also be brought about in the same way as that of non-living proteins such as egg-albumin.

Lepeschkin consequently comes to the conclusion that the plasma-membrane contains both protein and lipid substances in important quantities. He dismisses, however, the view of Nathansohn (1904 *a*) that the membrane consists of a mosaic of lipid substances and protein responsible respectively for the intake of lipid-soluble and water-soluble substances, since the entry of these two sorts of substances is not independent one of the other. Thus, the entry into the cell of salts and dyes soluble in water is diminished in the presence of narcotics soluble in fats. The value of this argument is, however, doubtful.

Lepeschkin's own view is that the plasma-membrane is largely composed of lipid substances loosely combined with protein.

It will be observed that both Czapek and Lepeschkin worked with vacuolate cells, and there is no evidence to decide whether they are dealing with the whole thickness of the protoplasm, or only a thin limiting layer of it. Lepeschkin recognised this quite clearly, simply using the term plasma-membrane to designate that part of the protoplasm which is the seat of the phenomena he investigated. This plasma-membrane is the surface layer and an unknown thickness of cytoplasm within it, even possibly the whole thickness.

For an account in English of the work of Czapek and Lepeschkin,

brief, but containing all the essential facts, reference may be made to an article by F. F. Blackman (1912).

Osterhout (1911, 1913 *a*) concluded that the plasma-membrane could not be lipoid in character because, contrary to the finding of Overton, Osterhout had shown that a large number of inorganic salts penetrate into the cells of *Spirogyra*. Further, although both sodium chloride and calcium chloride when presented in pure solution can enter *Spirogyra* cells, neither of these salts can penetrate into these same cells when present together in a solution in certain proportions. To this phenomenon of "antagonism" we shall return later. It will be sufficient here to remark that Osterhout regards his experiments as indicating a protein rather than a lipoid plasma-membrane.

In summing up the data here presented dealing with the composition of the limiting membrane of the protoplasm, it is clear that any view which regards the surface layer as composed exclusively of one kind of substance, either fatty or protein, must be dismissed at once. Both on theoretical grounds, and as the result of experimental work, it seems clear that the limiting layer of the cell must contain those constituents of the protoplasm and of the external liquid which lower the surface tension. These substances will thus constitute a colloidal complex, either a sol or gel, but how the molecules of the various constituents are arranged in reference to one another our present knowledge is insufficient to indicate.

Finally two points appear to deserve special emphasis. Firstly, on the view of the plasma-membrane here adopted, it is clear that the limiting membrane of the cell is not an invariable structure, but will vary with alterations in the composition both of the external medium and the protoplasm. It is clear that the hypothesis of de Vries, that the plasma-membranes are permanent structures never arising *de novo* but only from pre-existing membranes, is completely untenable.

Secondly, we can understand how it is impossible to separate the limiting membrane from the rest of the protoplasm. It depends for its existence on, and is closely related to, the media which it separates, and we should expect it to pass over into the two phases on either side of it, gradually in some cases, more or less sharply in others, according to the composition of the protoplasm and the external medium.

THICKNESS OF THE PLASMA-MEMBRANE

Seifriz (1921) has recently discussed this question. It is obvious that the plasma-membrane must be very thin, and that any measure-

ments must be very crude approximations. With this provision Seifriz concludes that the thickness of the plasma-membrane is of the same order of magnitude as that of precipitation membranes, namely 0.1μ (0.0001 mm.).

OTHER CELL MEMBRANES

Some of the arguments for the presence of plasma-membranes bounding the vacuole and the outside of the protoplasm hold equally for the presence of such membranes between the protoplasm and nucleus, and the protoplast and chromatophores or plastids. Kite (1913 *a*) indeed, who regards plasmatic membranes as hypothetical structures, regards the nuclear membrane as definitely present. He describes it as a stiff gel and not to be confused with hypothetical structures such as plasmatic membranes. Accepting for the moment the membrane theory of cell permeability, an experiment described by Osterhout (1913 *b*) illustrates very prettily how such membranes may differ in permeability from the plasma-membrane. Cells of the marine alga *Griffithsia* contain chromatophores which are pigmented with chlorophyll and a red pigment soluble in water. Under normal conditions the pigment remains in the chromatophores, it being assumed the chromatophore membrane is impermeable to it. If the cell is treated with certain concentrations of ammonium chloride, the vacuole contracts while the outer surface of the protoplasm "still retains its full turgidity." At a certain stage in this process the red pigment diffuses out from the chromatophores but remains within the protoplasm, which thus becomes stained deep red. This is held to show the differential permeability of the chromatophore-membrane and of the plasma-membranes to the red pigment. It must not be forgotten that it would be possible to explain the phenomenon on the basis of changes in colloidal complexes without the invocation of membranes with differential permeability.

LABORATORY NOTES

THE PROJECTION MICROSCOPE

BY H. J. DENHAM, M.A.

THE simple projection apparatus described by Professor Small¹ has undoubtedly several advantages over the usual form of Abbé drawing camera, particularly as regards the comfort of the worker, which becomes a matter of some importance when many drawings have to be made; at the same time, it has its limitations as regards critical resolution, and is not to be recommended for cytological or other preparations where differentiation in depth is important. A similar form is very largely used in American laboratories, simple outfits having been placed on the market some time ago by two large optical firms there, but the illuminant is usually a gas-filled concentrated-filament lamp, which does not compare with the Pointolite as a source of light: while on the continent the Leitz small "Edinger" outfit is used, in which the source of light is a small arc, and the microscope is inclined at an angle of 45° , which has advantages over the horizontal position when temporary preparations are examined.

There are two small improvements which might be made in Professor Small's apparatus. The addition of a condenser to parallelise the rays from the illuminant is essential if a critical image is desired, and makes it possible to place the Pointolite at some distance from the microscope, simplifying adjustments and allowing more room for light filters and cooling troughs when required. The 100 C.P. lamp emits considerable energy in the form of heat, and the writer finds that the 30 C.P. pointolite is much more useful in the botanical laboratory. The bulb is much smaller, allowing a shorter focus lens to be used, placed much closer to the incandescent bead, with consequent increase in the intensity of illumination.

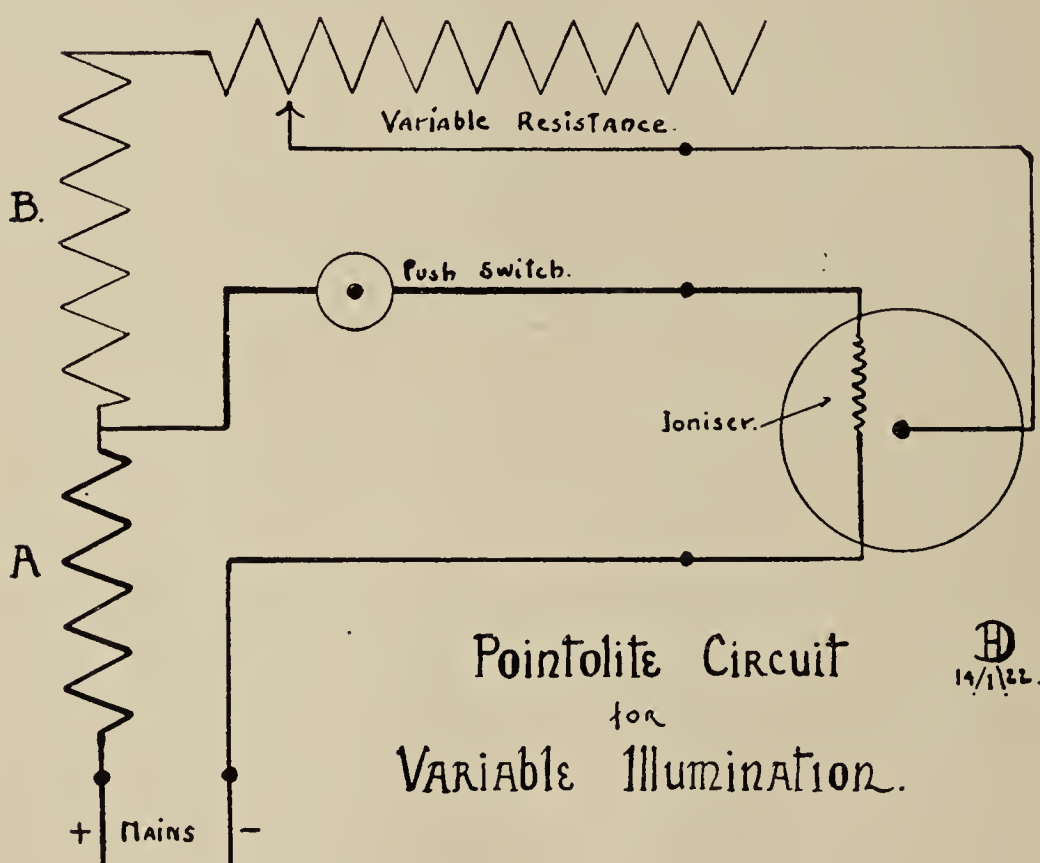
The second improvement, to obviate the necessity of a night-light, is the insertion of a variable resistance in the arc circuit of the Pointolite as shown in the figure. This has been described by the

¹ "Notes on an easy method of camera lucida drawing," *New Phytol.* 20, 1921, pp. 238-241.

writer¹ for normal microscope illumination, but works equally well for projection.

It is quite unnecessary to buy a special resistance for a Pointolite outfit, as these can be made up more simply and cheaply in any well-equipped laboratory. The published figures for the 100 C.P. are as follows:

Volts	Resistance in ohms	
	A	B
100	12	30.2
110	13.3	36.7
200	25.5	93.5
220	28	106.5
240	31	119



and the only part that must be purchased from the makers is the special three-contact lampholder.

The resistances for the 30 C.P. are not published, but can be constructed from the data that the ioniser circuit requires 4.5 amps. (for five seconds when starting), and the arc circuit 0.45 amp. A resistance supplied by the makers for 200 volts has a resistance of 42 ohms

¹ *Nature*, Nov. 17, 1921, p. 369, "Microscope Illumination and Fatigue."

for *A*, 295 ohms for *B*. This would indicate an ioniser resistance of 2.5 ohms, and an arc resistance of 107 ohms, but in any case the made-up resistance should be finally adjusted with the help of an ammeter under working conditions.

Much variation exists in the design of reflecting prisms and mirrors for use at the eyepiece, and several very unsatisfactory forms are on the market. The most useful pattern which the writer has seen is made by the Spencer Co., and consists of a right-angled prism attached to a rather long arm which is pivoted to a collar. This collar normally fits some way down the draw-tube of the microscope, and consequently makes it possible to employ such eyepieces as the Zeiss K. 12, which are of an awkward shape. Bausch and Lomb make a useful form which has the same advantage but also includes a Nicol prism analyser which can be swung out of the way when not needed.

The most convenient and efficient surface for the examination of projected images appears to be a good quality Bristol board, though a plaster of Paris surface has been recommended. If precautions are taken to exclude all extraneous light, by darkening the room and shielding the illuminant, quite satisfactory microphotographs can be taken by substituting a photographic plate or bromide paper for the drawing surface. Black light-proof fabric, such as velveteen, mounted on a wire or wooden frame, is preferable to cardboard for shielding. This form of camera is recommended as the simplest and at the same time the cheapest available, having regard to efficient working.

Under favourable conditions direct sunlight can be used, reflected by the substage mirror, but unless a heliostat is available, the mirror will require frequent alteration.

To avoid the possibility of the outfit being accidentally thrown out of adjustment, the components should be securely fastened to a stout baseboard.

DIDSBURY, MANCHESTER.

January 14, 1922.

A MULTIPLE SWITCH AND COMMUTATOR
FOR THERMOCOUPLES

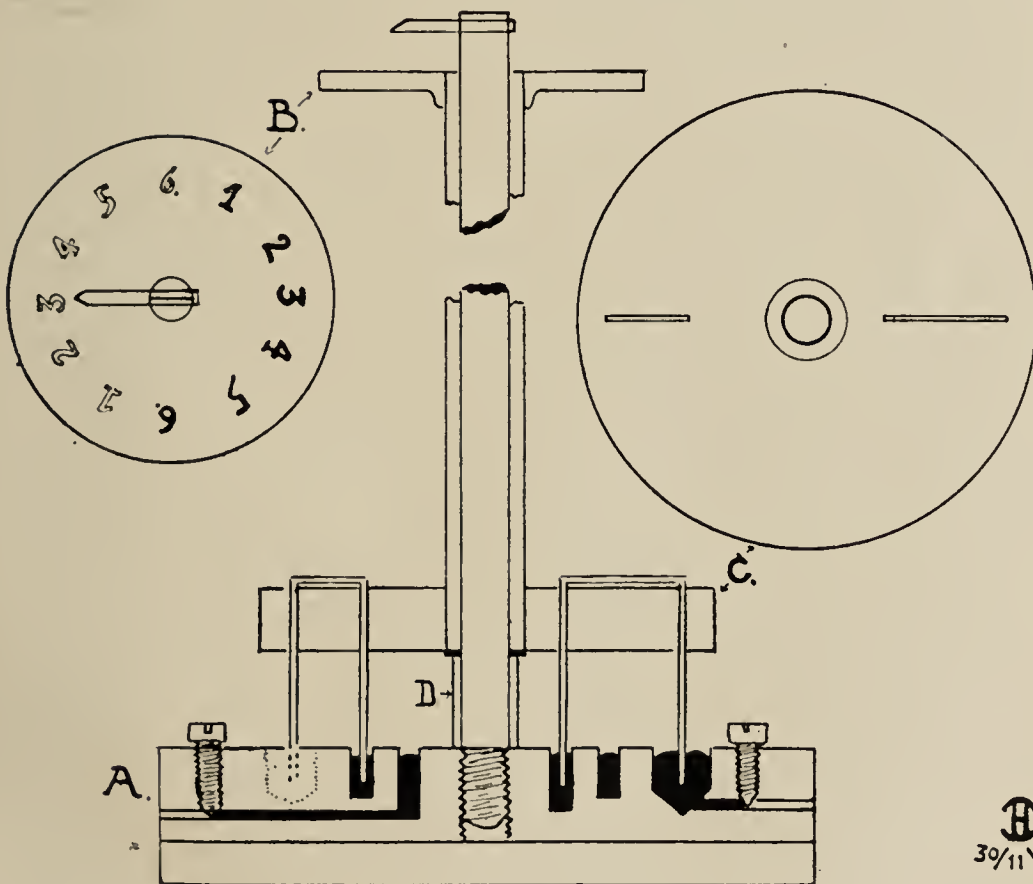
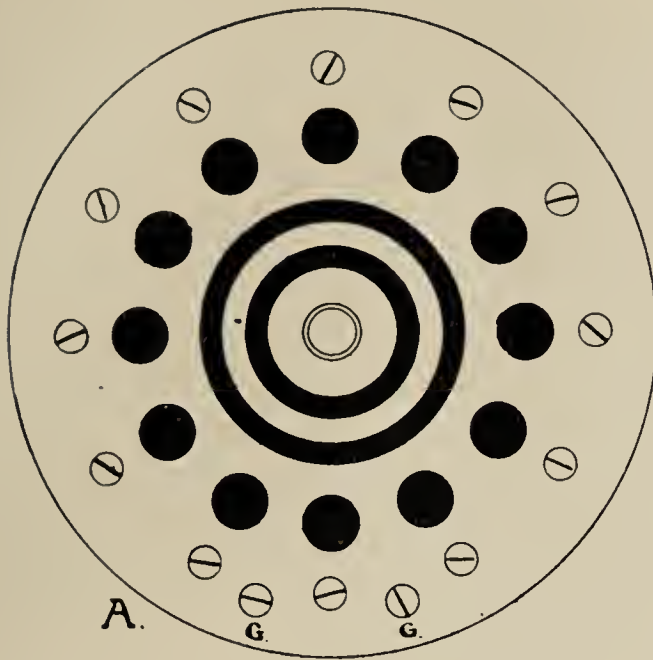
BY H. J. DENHAM, M.A.

IN the course of an experiment involving the use of several thermocouples with the same galvanometer, the need for some form of multiple switch was severely felt. A simple mercury switchboard was tried, but condemned on account of the thermo-electric errors which it introduced, and it became apparent that the switch must be worked at constant temperature and shielded from the warmth of the hand and other influences; accordingly, the rotary commutator described below was designed and constructed by the writer to work in an oil bath at constant temperature.

Description. The instrument (see figure) consists essentially of two parts: a base (*A*) of ebonite, containing twelve or more mercury cups, connected in diametric pairs to the thermocouple or other circuits, and two concentric grooves filled with mercury, connected to the galvanometer. The base is screwed at the centre to receive a metal stem long enough to reach well above the surface of the bath, and provided at the top with a removable pointer. On this stem works a sleeve of ebonite or glass, carrying at its upper end an engraved actuating head (*B*) of xylonite, and at its lower end a selector disk (*C*). This disk (whose function is to prevent sediment from falling into the mercury) is provided with two rigid bridge-pieces of wire, which serve to connect the concentric grooves to the mercury cups, and is supported above the base by a short metal sleeve and washer (*D*). Connections to the mercury cups and grooves are made by holes drilled horizontally in the base, of such a diameter as to take the bared wire of the leads, each hole being provided with a clamping screw which holds the wire in place.

Method of use. The switch should be installed in a bath provided with a stirrer and, if extreme accuracy be desired, a thermostat, the actuating head and pointer being allowed to stand well above the surface of the liquid.

The actuating head is lifted to disengage the contacts from the cups, and rotated until the number of the circuit required is opposite the pointer, when it is lowered and contact is made. To reverse the polarity of the circuit, the actuating head is again lifted and rotated through 180° , when the circuit number again comes before the pointer. To avoid confusion the two sets of numbers should be differently



engraved on the head, as in the figure, or filled in with red and black. Before use all contacts should be amalgamated. The use of iron or steel wire should be avoided, particularly when oil is used, as the system may act as a Lodge coherer.

Modifications. Where intermittent contact only is required, a spring may be fitted over the distance piece or sleeve (*D*), to lift the contacts out of the mercury when pressure on the actuating head is released.

When the instrument is required only for "dry" work, the long stem and actuating head may be dispensed with, and the numbers of the circuits engraved on the selector disk, which should be fitted with a central knob, and then serves as a temperature shield.

By fitting the selector disk with bridge-pieces other than those shown, the switch may be used for a very large number of combinations and complex circuits. The number of mercury cups is limited only by the size of the base.

SHIRLEY INSTITUTE,
DIDSBURY, MANCHESTER.
November 30, 1921.

THE NEW PHYTOLOGIST

VOL. XXI, No. 4

NOVEMBER 7, 1922

PERMEABILITY

By WALTER STILES

CHAPTER IX

THE WATER RELATIONS OF THE PLANT CELL

INTIMATELY connected with problems of cell permeability are the water relations of the cell. Already in the preceding chapter in considering the evidence for the existence of plasma-membranes it has been necessary to touch on this subject. It has there been indicated that the evidence for the existence of a semi-permeable membrane forming the limiting layer of the protoplasm is very conflicting. Hence in the case of the non-vacuolated cell it is not at all clear whether it is correct to think of the cell with regard to its relations to water simply as an osmotic cell, that is, as a solution of osmotically active substances enclosed in a semi-permeable membrane, or whether we are to compare it rather with a colloidal system such as a gelatine or agar-agar gel or blood fibrin. It is true that M. H. Fischer (1908, 1910, 1915) has shown that certain animal tissues (frog's muscle and sheep's eyes) behave similarly to gelatine and blood fibrin with regard to the absorption of water, while MacDougal (1916 *a*, 1916 *b*, 1917, 1918, 1920, 1921) and MacDougal and Spoehr (1917 *a*, 1917 *b*, 1917 *c*, 1919, 1920) have succeeded in preparing colloidal mixtures of agar-agar with gelatine, albumin or other proteins, urea or amino-acids, in which agar-agar forms at least three quarters of the whole, the so-called bio-colloids, which are very similar to plants in their behaviour towards water. Lloyd's work indicating a similar conclusion has been referred to in the last chapter. But while we may be sure that living plant protoplasm will imbibe water in the same way as

a number of gels and other colloids, the evidence that this will completely account for the water relations of the non-vacuolated cell lacks definiteness. A considerable amount of quantitative work is necessary both on non-vacuolated plant cells and on colloidal systems before the question can be regarded as in any way settled.

Similarly in the vacuolated cell, where the evidence that we have to do with a system constituting an osmotic cell is more conclusive, it is not at all clear that it is correct to regard the limiting layers of the protoplast as semi-permeable membranes separating the protoplasm on the one side from an external solution contained in the cell wall, and on the inner side from the liquid in the vacuole. On the other hand, in the present state of our knowledge it does seem reasonable to suppose that the whole layer of protoplasm may act as a membrane separating vacuole and cell wall, and to some extent semi-permeable inasmuch as it may allow the ready passage of water, while varying greatly in its permeability to different substances dissolved in the water, and even completely preventing the passage of some.

TURGIDITY

Whether on account of the swelling of the colloidal constituents of the cell, or because the cell behaves as a solution of crystalloids surrounded by a semi-permeable membrane, or on account of both these reasons, under most circumstances the cell will absorb or has absorbed water to such an extent, that it exerts a pressure on the cell wall, which being capable of extension, is stretched. But as the cell wall is elastic, that is, tends to return to its original form on being stretched, it exerts a pressure against its extension, and consequently against the increase in volume of the cell. The cell is therefore only capable of absorbing water until the inwardly directed pressure resulting from the stretched condition of the cell wall is equal to the pressure sending water into the cells. The rigidity of the cell resulting from this state of affairs has been not inaptly compared with that of an inflated bicycle tyre or football (*e.g.* by Livingston, 1903). It is spoken of as turgidity¹, while the cell is said to be turgid.

¹ The term "turgescence" is sometimes used to express the same condition of the cell (*e.g.* de Vries, 1874, and many later writers). The term "turgor" is sometimes used in the same sense (*e.g.* by Gager, 1916). This term is sometimes used to express a definite quantity, but it is perhaps best to limit it to its qualitative sense.

QUANTITATIVE RELATIONS OF THE CELL IN REGARD
TO WATER ON THE SIMPLE "OSMOTIC" VIEW

Omitting from our consideration the complications introduced if the protoplasm is in fact separated from cell wall and vacuole by semi-permeable membranes, which, in the absence of definite evidence, we are at least partly justified in doing, it is clear from what has been said in preceding chapters that in a full consideration of the permeability of the vacuolated plant cell to water we have to take into account the water relations of three distinct phases: the cell wall, the protoplasm and the vacuole.

It is generally assumed that the cell wall is completely permeable both to water and substances dissolved in the water, while it appears to be accepted that the protoplasm is readily permeable to water, but is impermeable or permeable in varying degrees to dissolved substances. The only part the cell wall is supposed to play in the water relations of the cell is to act as an elastic covering as described above, while the protoplast acts as a semi-permeable membrane enclosing the solution of salts and other crystalloids in the vacuole. This view of the cell I would speak of as the simple osmotic view, for it regards the living cell in its water relations as a simple osmotic cell. From what has been said in earlier chapters of the constitution of the protoplasm and cell wall it is obviously a considerable simplification of the actual state of affairs, neglecting as it does absorption of water and salts by the cell wall and protoplasm, with consequent changes in properties of these essential parts of the system, together with all electrical effects such as negative osmosis. Nevertheless the simple osmotic view is the one generally taken in discussions on the water relations of the plant cell, the reason for this being no doubt partly a natural preference for the simple rather than for the complex, partly to the beautiful work of de Vries (1884 *a*, 1885) which did so much to render the view popular, but no doubt also because in many cases the assumptions involved do not introduce errors of any considerable magnitude. This will most probably be the case where the cell wall is thin and the volume of the protoplast very small in comparison with that of the vacuole.

The simplest case we can consider is that of the single isolated cell. This is to be regarded as a solution of crystalloids (the cell sap) surrounded by a semi-permeable membrane (the protoplast), itself surrounded by the elastic cell wall. In the discussion immediately following it is assumed that the protoplast is absolutely semi-

permeable, a state of affairs rarely realisable in practice. When such a cell is immersed in a solution it is assumed that the latter permeates the cell wall so that the concentration of the solution in the cell wall is the same as that in the external solution. If the cell wall were absent, or if it were indefinitely extensible but completely inelastic, water would pass across the semi-permeable membrane until the difference in osmotic concentration on the two sides of the semi-permeable membrane is zero. With water or very dilute solutions forming the external medium, water will pass into the cell in some cases until the latter bursts owing to the attenuation of the protoplasm, as we have already noted in the preceding chapter.

But with the presence of the elastic cell wall another force comes into play. We have already noticed that as the cell swells with absorption of water, the stretched cell wall exerts an inwardly directed pressure increasing with increase in stretching of the wall, and opposing the passage of water into the cell resulting from the difference of osmotic concentration on the two sides of the protoplasmic membrane. In the case of the turgid cell equilibrium will therefore be reached when the force sending water into the cell, that is, the difference in osmotic pressure of the internal and external solutions, is equal to this inwardly directed pressure exerted by the stretched cell wall. Before equilibrium is reached the pressure forcing water into the cell, that is, the difference in osmotic pressure of the internal and external solutions, is only partly compensated by the internally directed pressure of the stretched cell wall, so that the net pressure sending water into the cell is the difference in osmotic pressure of internal and external solutions less the inwardly directed pressure of the stretched cell wall. This net pressure obviously gets less and less as the difference in osmotic pressure inside and outside the cell is less and the pressure exerted by the wall greater.

The terminology relating to these water relations of the turgid cell is unfortunately somewhat confused. It will be noted that we are concerned with the following quantities. There is firstly the osmotic pressure of the external solution. This may be expressed in terms of molecular solutions of sucrose or potassium nitrate, or in the corresponding atmospheric pressure. Secondly, there is the osmotic pressure of the liquid in the vacuole, the cell sap. For this quantity a number of terms have been employed such as osmotic pressure, concentration of the cell sap, potassium nitrate or saltpetre value, turgor, turgor pressure and others. The term used here will be osmotic pressure when it is the pressure sending water into the cell

to which reference is made; but as the osmotic pressure can only exist when the solution is separated from another or from pure water by a membrane, a term is useful to indicate the concentration of substances in the cell sap in terms of the osmotic pressure that would be produced when such a solution is separated from pure water by a semi-permeable membrane. For this quantity the term osmotic concentration may be employed. Ursprung and Blum (1916 *a*) prefer to denote this quantity by the term "osmotic value," which also appears free from all academic objections.

Thirdly, there is the inwardly directed pressure of the cell wall (wall pressure) which is equal and opposite to the hydrostatic pressure exerted against it by the liquid in the cell. This hydrostatic pressure has also been spoken of as turgor or turgor pressure, but it will be observed that it is not the same quantity as the osmotic pressure for which the term is sometimes used. Here the term turgor pressure will be used to designate the total hydrostatic pressure exerted by the cell liquid against the protoplast and cell wall.

Fourthly, there is the net pressure sending water into the cell. This, as we have seen, is equal to the difference between the osmotic pressure of the external solution and the osmotic pressure of the cell, less the turgor pressure. This quantity is called by Ursprung and Blum (1916 *a*, 1916 *d*, 1916 *e*) and other writers in German (*e.g.* Höfler, 1920), often with the assumption that the external liquid is water, the suction force ("Saugkraft"), a term that has been applied to the net pressure sending water into whole organs and tissues. As this quantity is a pressure rather than a force it will here be termed the suction pressure (Stiles, 1922), and it will be convenient to distinguish between the net suction pressure, whatever the external liquid, and the full suction pressure¹ when the external liquid is water.

¹ Ursprung and Blum (1916 *d*) clearly recognise the dimensions of the suction pressure as being those of a pressure, but for reasons which do not appear very forceful prefer to retain the term "Saugkraft." They say, "Was die Terminologie betrifft, so soll die Bezeichnung 'Kraft' beibehalten werden, obschon es sich ja um $\frac{\text{Kraft}}{\text{Fläche}}$ handelt, also um eine Grösse die nicht in Kg sondern in Atm. gemessen wird. Man pflegt ja auch in der Physik von Zug- und Druckkräften zu reden." Nor has the term "water absorbing power" proposed by Thoday (1918 *b*) for this quantity much to recommend it, for the quantity is certainly not a power but a pressure, and the term suction force has the claim of priority and the advantage of brevity, and in the form of suction pressure appears to be free from all disadvantages. When the term "suction pressure" alone is used in the following, it indicates the full suction pressure.

If P is the osmotic pressure of the liquid in the vacuole, P_e the osmotic pressure of the external solution, T the inwardly directed pressure exerted by the cell wall, and if S is the net suction pressure, the relation between these quantities is given by

$$S = P - P_e - T,$$

or, if the external liquid is pure water, so that $P_e = 0$,

$$S = P - T.$$

This relation was fully realised by de Vries (1884 *a*) who definitely states that the water attracting force of turgid tissue is not the same as that of the cell sap contained in its cells, but is smaller by a quantity corresponding to the elastic tension force of the protoplast and the cell wall.

When a cell is in equilibrium with pure water the turgor pressure is at its highest possible value; replacing the water with an osmotically active solution, for example, that of a sugar or a salt, must result in the passage of water from the cell as the equilibrium is disturbed by the increase in the osmotic pressure of the solution outside the cell. Consequently the turgor pressure is less and the cell comes to occupy less volume. With progressive increase in the concentration of the external solution the protoplast will contract and the cell wall along with it until a point is reached in which the cell wall no longer contracts along with the protoplast, so that a space appears between cell wall and protoplasm. As it is assumed that the cell wall is completely permeable both to water and dissolved substances, this space is filled with the external solution so that the unextended cell wall no longer exerts any pressure on the protoplast, the cell is flaccid and the turgor pressure is zero. The suction pressure in such plasmolysed cells is simply proportional to the difference between the osmotic pressures of internal and external solutions, and when equilibrium is attained the osmotic pressure of the cell sap is the same as that of the internal solution.

It has already been noted that when the cell is in equilibrium with water the turgor pressure is at its highest possible value, and the suction pressure (at equilibrium) being zero, the turgor pressure is equal to the osmotic pressure of the cell. At the beginning of plasmolysis the turgor pressure has reached its minimum value, that is, zero. Between these limits, the turgor pressure possesses intermediate values depending on the quantity of water taken into the cell. It has generally been assumed that within the limits of elasticity of the cell wall the tension of the wall increases proportionately

to the volume of water taken into the cell (Nägeli and Schwendener, 1877; Pfeffer, 1873; Thoday, 1918 *b*). Ursprung and Blum (1916 *d*) have shown, however, that such a relation only holds approximately. They point out that Sacerdote (1898) has shown that for a hollow cylinder with thin walls the increase in volume δV of the original volume V is given by the equation

$$\frac{\delta V}{V} = -3a \left[(1 - 2\sigma) p' - (1 - \sigma) \frac{R}{2e} (p - p') \right],$$

where R is the radius of the cylinder, e the thickness of the wall, p and p' the pressures on the inner and outer sides of the wall, V the volume and a and σ are constants.

When R/e is very large the first term on the right side of the equation may be neglected, and in this case the increase in volume will be proportional to the increase in pressure. But if the first term is not negligible in comparison with the second, the linear relation between wall pressure and volume is only approximate. Diagrammatic representations of this linear relation are given by Thoday (1918 *b*) and Höfler (1920).

Assuming this linear relation, if V_z is the volume of the cell when the turgor pressure is zero, but before plasmolysis has commenced (the state referred to by Ursprung and Blum as "Grenzplasmolyse"), and if the osmotic pressure is P_z , and if when the turgor pressure (or wall pressure) is T the volume of the cell is V and its osmotic pressure P , neglecting the volume of the protoplasm, and assuming constant temperature, the following simple relations hold:

$$P_z V_z = PV \quad \text{.....(Cf. Chapter VI),}$$

$$\frac{V - V_z}{V} = aT,$$

where a is a constant. The ratio V/V_z Höfler denotes by the term "degree of turgor stretching¹."

If the volume of the protoplasm of the cell is not negligible in comparison with the volume of the vacuole, these equations require modification. If p represents the fraction of the whole volume of the cell in the non-turgid but unplasmolysed condition occupied by the protoplasm, and if the latter undergoes no change in volume

¹ Höfler points out that the relation between osmotic pressure of the cell and turgor pressure is incorrectly given by Pantanelli (1904) and Höber (1914) following him.

during the intake of water by the vacuole, the former of the two equations just given becomes

$$P_z V_z (1 - p) = P (V - p V_z),$$

or, if v is the degree of turgor stretching,

$$P = P_z \frac{1 - p}{v - p},$$

the form in which the relation is given by Höfler.

Should the volume of the protoplasm also change with increasing turgor of the cell, this equation has to be further modified. In the absence of information on this point, however, further elaboration of this question is scarcely profitable.

It is to be observed that the relations given above only hold within the limits of elasticity of the cell wall. It must be admitted that we know very little about the elasticity of the cell wall. Schwendener and Krabbe (1893) immersed cylinders of young pith cells in water with the result that they swelled so that the length increased 25 to 30 per cent. On plasmolysis they contracted to the same length as that of similar cylinders plasmolysed immediately after their isolation from the plant without any immersion in water before plasmolysis. According to Pfeffer (1873) the staminal filaments of *Cynareæ* can be stretched to double their normal length without the limits of elasticity being exceeded. On the other hand, some cells, including bast fibres, are incapable of elastic stretching exceeding 0.5 to 1.5 per cent. of their normal length (Pfeffer, 1903).

Although a considerable amount of work has been done on the question of the elasticity of the cell wall, yet owing to the complex systems which constitute plant tissues, such work is full of difficulties and the results obtained open to considerable criticism. A discussion of this is out of the question here, but it may be pointed out that after considerable stretching the cell wall may take some time to return to its original shape, recovery being gradual (Detlefsen, 1884, 1888), while the modulus of elasticity will vary with the water content of the cell wall (Pfeffer, 1903). It is clear that relations involving assumptions with regard to the elasticity of the cell wall should be accepted with caution.

ISOTONIC SOLUTIONS AND ISOTONIC COEFFICIENTS

A solution having the same osmotic concentration as the cell sap is said to be isotonic with it, a solution of greater concentration than the cell sap is described as hypertonic, and one of lower concentration than the cell sap as hypotonic.

In the case of a cell without a wall in which it is assumed that the resistance to stretching offered by the protoplast is negligible, a cell immersed in a solution isotonic with the cell sap will undergo no change in volume, while in a hypertonic solution contraction, and in a hypotonic solution increase in volume of the cell will take place until the osmotic pressure of the liquid on the two sides of the protoplasmic membrane is the same.

In the case of turgid cells we have already noted that the suction pressure is less than the difference in the osmotic pressures by the turgor pressure, so that when, after immersion in a solution, no change in volume occurs in a turgid cell, the external solution is hypotonic, the tendency for water to enter the cell on account of the difference in osmotic pressure, being just balanced by the turgor pressure tending to force water out.

If now, a turgid cell is immersed in a solution of such a strength that a contraction of the protoplast away from the cell wall is just, but only just, brought about, the osmotic pressure of the solution external to the cell will be equal to that of the cell sap at the moment when the turgidity of the cell just disappears. It will be observed that the osmotic pressure will be somewhat greater than that of the turgid cell, as the latter has a somewhat greater volume than the cell at the commencement of plasmolysis, and consequently, as the total amount of solute in the cell remains unaltered, the osmotic pressure of the liquid in the vacuole must increase as the vacuole contracts.

If the concentrations of a number of different substances which just bring about plasmolysis of similar cells are determined, these different solutions should have the same osmotic pressure, and are therefore described as isosmotic. De Vries (1884 *a*) regarded such solutions as having the same attraction for water, so that the attraction of a molecule of any one substance for water would be inversely proportional to the concentration of its isosmotic solution. By the term *isotonic coefficient* of a substance de Vries (1884 *a*, 1888, 1889) signified the magnitude of the attraction of a molecule of the body in dilute aqueous solution for water. As unit he took one-third the attraction of a molecule of potassium nitrate.

De Vries concluded that the isotonic coefficient in strongly diluted solutions is a constant for every compound, the constant depending in a certain way on the composition of the compound and being expressible approximately by a simple number. The isotonic coefficients found by de Vries are given in the following table.

TABLE XVIII

Approximate Isotonic Coefficients of de Vries

Substances	Isotonic coefficient
Organic metal-free compounds	2
Alkali salts with one metallic atom in molecule ...	3
" " two " atoms " ...	4
" " three " atoms " ...	5
Alkaline earth salts with one metallic atom in molecule	2
" " " two " atoms "	4

The value of the isotonic coefficient depends, of course, on the osmotic pressure, which depends, as pointed out in Chapter VI, on the degree of ionisation of the solution, the high values of the isotonic coefficients of electrolytes as compared with organic compounds such as sugar being explained on this ground. Since, as pointed out by Livingston (1903), the coefficients are only true values within limits (cf. Chapter VI), and as more accurate methods are available for the determination of the degree of ionisation, we may agree with Livingston that "it will be well for physiology when the practical use of these coefficients dies out entirely." Nevertheless, the work of de Vries on this subject was nearly as important for both physiology and physical chemistry as that of Pfeffer on osmotic pressure, and diagrams of unplasmolysed and plasmolysed cells taken from de Vries are to be found in standard text-books of botany and physics (*e.g.* Poynting and Thomson, 1905). Moreover, the methods elaborated by de Vries (1884 *a*, 1884 *b*) for the determination of isotonic coefficients have found a wide use for physiological problems connected not only with the water relations of the cell, but also with the passage of dissolved substances into and out from the cell. Some description of these methods is therefore desirable. They are the plasmolytic methods (the comparative plasmolytic method and the plasmolytic transport method) and the method of tissue tension.

Comparative plasmolytic method. Certain experimental conditions are desirable for the successful working of this method. These are (1) that the smallest trace of plasmolysis should be easily recognisable, (2) that all the cells of the tissue used should just plasmolyse in solutions of the same salt of exactly the same concentration, and (3) that the substances examined should not penetrate the cells. De Vries specially recommended three particular kinds of cells for this work, namely (1) the epidermal cells of the outer side of the growing leaf sheath of the dark red form of *Curcuma rubricaulis*, (2) the violet cells of the under epidermis at or near the midrib of

leaves of *Tradescantia discolor* (*Rhæo discolor*), and (3) red cells of the upper epidermis of the scales of the leaf stalk of *Begonia manicata*, together with cells of the red spots in the epidermis of the leaf stalk in the neighbourhood of the scales. The last is not to be recommended so much as the other two, but is particularly useful in the case of acids. The chief advantage of these particular cells is that they contain coloured sap in the vacuole which renders the observation of the slightest sign of plasmolysis much easier than in the case of cells with colourless sap.

It is a disadvantage of this method that individual cells are observed, and if different cells of the same tissue differ in their osmotic pressure observation has to be made on a number of cells. In order to reduce differences in osmotic pressure of different cells to a minimum, it is necessary to use cells as near together as possible. If the cells are not all of the same osmotic pressure, the solution in which half the cells become plasmolysed may be taken as the solution which is osmotically equivalent to the mean of the cell saps (cf. Darwin and Acton, 1901). A slight disadvantage is that the method requires time as the observer has to wait for equilibrium to result which is not likely to take less than an hour. An advantage of the method is that material for its performance can be obtained at all seasons of the year. The exactness of the determination depends on the degree of agreement between the different cells employed, on the number examined to determine the mean osmotic pressure of the cells and on the differences in concentration between the solutions of any of the substances examined.

Plasmolytic transport method. In this variant of the plasmolytic method cells are weakly plasmolysed in a solution of the salt under investigation and when plasmolysis is ended the cells are transferred to potassium nitrate solutions of different concentrations. That solution in which the volume of the plasmolysed protoplast remains unchanged is isotonic with the solution first used. This method has the advantage as compared with the comparative plasmolytic method that the same cell is used for the comparison of the experimental solution with the standard ones. It has the disadvantage that the long immersion of the cells in salt solutions may result in damage to the cells. De Vries regarded the method as useful chiefly in the investigation of the influence of concentration on isotonic coefficients, as the method allows the use of a range of concentrations.

Method of tissue tension. For the practice of this method a piece of the growing stem or other elongated organ is cut out from a plant

and split longitudinally into four parts. Usually the pith elongates and the epidermis contracts owing to tensions in the tissue; consequently the strips of tissue curl with the pith occupying the convex side of the strip. If put into water the pith takes up water rapidly so that the whole rolls up into a spiral. If put in a strong enough salt solution the pith gives up part of its water so that the pith contracts and the curvature is reduced or may even be reversed, that is, the pith occupies the concave side. A solution of any particular substance can thus be obtained by sufficiently exact grading of the solutions in which no change of curvature takes place at all. In this concentration in which water is not taken up, the osmotic pressure of the external solution is equal to the osmotic pressure of the cells less the turgor pressure of the cells. Since these two last quantities may be assumed the same in similar strips of the same tissue, it follows that the solutions of different substances which just bring about no intake or excretion of water from the pith are isotonic with one another, though not with the cell sap. This method has two advantages. As a great number of cells are involved the values obtained are mean values. Also the experiment only takes a few minutes; indeed a long duration of the experiment must be avoided in order to prevent absorption of the salt or other dissolved substance by the tissue. According to de Vries it is a disadvantage of this method that the material for it can only be obtained in spring and summer, but there should be little difficulty in obviating this disadvantage where a warmed greenhouse is available. Details of the method in which scapes of dandelion (*Taraxacum dens-leonis*) are used as the experimental material in summer and hypocotyls of *Ricinus* seedlings are used in winter, are given in Darwin and Acton's *Practical Physiology of Plants* which is probably easily accessible to nearly all English readers¹.

¹ Darwin and Acton and other writers assume that the solution which produces neither increase nor decrease in curvature equals the cell sap in osmotic "force" (*i.e.* pressure), but as we have already seen, and as de Vries pointed out in his own description of the method, the osmotic pressure of the external solution which produces no change in the tissue is not equal to the osmotic pressure of the cell, but to the osmotic pressure of the cell less the turgor pressure. In the tissues used for the tissue tension method it does not follow that the turgor pressure is small enough to be neglected. This is immaterial in the determination of isotonic coefficients, for the osmotic pressures of the solutions of the different substances compared are in all cases presumed equal to the same osmotic pressure of cell sap less the same turgor pressure, and are therefore equal to one another. But the method does not give the osmotic strength of the cell sap, at any rate exactly. Thoday (1918 *b*) has accused Stiles and Jørgensen (1917 *b*) of falling into the same error when they speak of a solution in which disks of potato neither lose nor gain weight as

In Table XIX are given the exact values for the isotonic coefficients of a number of substances found by de Vries by the plasmolytic and tissue tension methods.

TABLE XIX
Isotonic Coefficients of a Number of Substances
(Data from de Vries)

Substance	Isotonic coefficient found by	
	Plasmolytic method	Tissue tension method
Sucrose	1.88	1.84
Malic acid	1.98	—
Tartaric acid	2.02	—
Citric acid	2.02	—
Sodium nitrate	3.0	—
Potassium chloride	3.0	2.84
Sodium chloride	—	3.05
Ammonium chloride	3.0	—
Potassium acetate	3.0	—
„ dihydrogen citrate	3.05	—
„ oxalate	—	3.93
„ sulphate	3.9	3.92
„ monohydrogen phosphate	—	3.96
„ tartrate	—	3.99
„ malate	—	4.11
„ monohydrogen citrate	4.08	—
„ citrate	5.01	4.74
Magnesium malate	1.88	1.63
„ sulphate	1.96	1.78
„ citrate	3.88	3.53
„ chloride	4.33	—
Calcium chloride	4.33	—

DETERMINATION OF THE OSMOTIC PRESSURE OF THE CELL

Accepting the assumptions already made with regard to the water relations of the cell, the determination of the osmotic pressure of the cell sap is a simple matter. The method usually employed is the plasmolytic method of de Vries already described; in a second method, recently proposed and used by Höfler (1917), the osmotic pressure is also measured in the cell as such; other methods in which the osmotic pressure of the expressed sap is measured either directly

“approximately isotonic with the cell sap.” But in such tissue as the potato tuber employed the turgor pressure is low in comparison with the osmotic pressure of the cell sap, and the solution in which such tissue neither gains nor loses weight is actually approximately isotonic with the cell sap (cf. Stiles and Jørgensen, 1919). With Thoday's “rejoinder” to this (1919), that it is incorrect to call a turgorless tissue turgescient, the joint authors would doubtless agree, at the same time pointing out that it is not they, but Thoday, who has suggested that the potatoes used had a high turgor pressure, the word turgescient not even appearing in any of their papers.

or by one of the indirect methods indicated in Chapter VI have also been much employed for determining the osmotic pressures of plant cells, as, for example, by Dixon and Atkins (1910-1916), Harris and co-workers (1916-1921) and Mason (1919). These various methods will now be briefly described.

Plasmolytic method. This method is that of de Vries for determining isotonic coefficients and has already been described. Solutions of sucrose and potassium nitrate are the media generally used to produce plasmolysis of the cells investigated. The solution which just brings about plasmolysis, or that which just fails to bring it about, is regarded as isotonic with the cell sap, and accordingly has the same osmotic pressure. The values of osmotic pressure of solutions of sucrose over a wide range of concentrations have been obtained by Berkeley and Hartley and by Morse and his collaborators, and the existence of these determinations renders sucrose particularly useful as a plasmolysing substance in the plasmolytic method for the determination of the osmotic pressure of plant cells. A table of these values is given by Ursprung and Blum (1916 *a*). Sucrose is also to be recommended as it enters the majority of cells with extreme slowness and so does not materially affect the determinations on account of increased concentration of the cell sap resulting from endosmosis.

Immersion of the cells for some time is necessary for plasmolysis to be observed in many cases. Ursprung and Blum (*l.c.*) left herbaceous parts of a number of plants immersed in the solutions for 25 to 40 minutes or longer. Sections of stem and root of *Fagus* required at least 40 minutes. This rather long immersion involves the possibility of concentration changes in the cell sap owing to exosmosis from the interior into the surrounding solution, so that an error may arise on this account. As plasmolysis and deplasmolysis may result in tearing and stretching of the protoplasm it is advisable to use fresh cells for every determination. Change in volume which the cell may undergo when the tissue examined is isolated from the plant may also influence the result and should certainly be looked for and taken account of, and the same remark holds with regard to change in the volume of the tissue taking place during its immersion in the solution. The value of the osmotic pressure obtained is that of the cell at the moment when plasmolysis commences. If the osmotic pressure of the external solution is then P_z , the volume of the tissue V_z and its original volume in the turgid condition V , its osmotic pressure in that condition is $P_z V_z / V$.

A great advantage of the plasmolytic method lies in the fact that it allows the investigation of the osmotic pressure of individual cells; when the mean value of all the cells of a piece of tissue is required the concentration isotonic with the mean cell sap may be reckoned as that in which half the cells examined show definite plasmolysis.

Plasmometric method. This method is due to Höfler (1917); it is essentially a variant of the plasmolytic method, but in some cases, at any rate, is easier in execution. The essentials of the method are as follows. If a non-turgid but unplasmolysed cell is plasmolysed in a decidedly hypertonic solution, its volume, when plasmolysis is complete, is reduced by a definite fraction of the original volume, let us say by $1/n$. Assuming complete semi-permeability as in the case of the plasmolytic method, the concentration of the cell sap must have increased to $n/n - 1$ of its original value. Then if V_p is the volume of the plasmolysed protoplast and vacuole, V_z the original internal volume of the cell, P_e the osmotic pressure of the plasmolysing solution, the osmotic pressure of the cell is given by

$$P_z = P_e \frac{V_p}{V_z} \quad \dots\dots(1),$$

and similarly if the cell was originally in a turgid condition with a volume V , the osmotic pressure is given by

$$P = P_e \frac{V_p}{V} \quad \dots\dots(2).$$

The ratio V/V_z is the degree of turgor stretching (cf. p. 175) and so the last equation can be written

$$P = \frac{P_z}{G} \quad \dots\dots(3),$$

where G is the degree of turgor stretching.

If it is assumed that the protoplast does not take part in the plasmolytic contraction the first and third of these equations become modified respectively to

$$P_z = P_e \cdot \frac{\frac{V_p}{V_z} - p}{1 - p} \quad \dots\dots(4),$$

$$P = P_z \cdot \frac{1 - p}{G - p} \quad \dots\dots(5),$$

where p is the proportion of the whole volume of the unplasmolysed but non-turgid cell occupied by the protoplast. Should the proto-

plast change its volume by a fraction αp the first equation becomes

$$P_z = P_e \cdot \frac{\frac{V_p}{V_z} - \alpha p}{1 - \alpha p}.$$

The quantity V_p/V_z is called by Höfler the degree of plasmolysis. In cells of regular geometric form in which there is a regular contraction on plasmolysis, both V (and V_z) and V_p are easily measured. In measuring the original volume of the cell it is necessary to measure the cell in a medium in which the cell undergoes no change in volume. For this purpose water and air are both ruled out, for in the former swelling may occur owing to intake of water, and in the latter evaporation of water may take place resulting in a diminution of volume. In air, moreover, the actual measurement is not easy. Ursprung and Blum (1916 *d*) recommend the use of liquid paraffin ("paraffin oil") for this purpose, as this substance neither reacts with nor enters the cell (Heller, 1904; Schilling, 1915). The cover glass must always be supported so that its weight does not fall on the cells or tissue and induce changes in form. The replacement of the paraffin by the plasmolysing liquid may be effected by irrigating the material on the slide, or by removing the material from the slide and washing in water. The former involves several successive washings to rid the cells or tissue of the paraffin, while the latter requires care to avoid deformation of the cells. Sucrose is to be recommended as the most generally useful plasmolysing agent for the reasons already given.

In a series of experiments in which parenchymatous cells from the stem of *Tradescantia elongata* were used, Höfler showed that the degree of plasmolysis was inversely proportional to the concentration of the plasmolysing solution, in the case of sucrose solutions varying in concentration from 0.30 to 0.60 gram-molecules a litre. With a number of solutions of different concentrations consistent values for the osmotic pressure of the cells were obtained, thus providing grounds for confidence in the correctness of the method.

Methods involving the determination of the osmotic pressure of the expressed sap. Many determinations of the osmotic pressure of the liquids in plant cells have been made by expressing the sap from the tissues and then determining the osmotic pressure of the sap by one of the methods mentioned in Chapter VI. The first point that calls for attention here is the method of extracting the sap from the tissues. Until comparatively recent the practice was to press the sap from the living untreated tissue (cf. Maquenne, 1896; Sutherst, 1901;

Cavara, 1901, 1905; Nicolosi-Roncati, 1909; Dixon and Atkins, 1910, 1912 *a*, 1912 *b*, 1912 *c*; Atkins, 1910; Marie and Gatin, 1912). A number of observations made by Dixon and Atkins among their earlier determinations led these authors to doubt the correctness of the assumption that the liquid so obtained represents an average sample of the sap. It had also been shown by André (1906 *a*, 1906 *b*, 1907 *a*, 1907 *b*) and by Marie and Gatin (1912) and confirmed by Dixon and Atkins (1913 *a*) that if a number of samples of sap are successively pressed from the same tissue, the samples exhibit a progressive increase in concentration and osmotic pressure. The explanation is that when pressure is first applied the protoplasm is not very permeable to the solutes in the vacuole, so that the first sample of liquid contains a much higher percentage of water than the vacuole. But the pressure results in the rupture of the cells and the passage out of the dissolved substances as well as the water. Consequently, as the pressure is increased to produce the successive samples of sap, more and more cells are burst so that the expressed sap becomes more and more concentrated.

In order to obtain an average sample of cell sap it is therefore necessary to treat the tissue so that the protoplasm is rendered completely permeable. Heat and toxic agents (toluene vapour and chloroform vapour) were tried but it was decided that the time required to render the cell membranes completely permeable was so long that enzyme or other actions might have sufficient play in the cells to alter materially the composition of the sap and so vitiate the results. Dixon and Atkins came to the conclusion that the most satisfactory way of rendering the cells completely permeable is to immerse the tissue in liquid air, as at this low temperature enzyme actions and autolytic and other changes are completely negligible. After such treatment the sap from successive pressings of the same piece of tissue had practically the same osmotic pressure. Subsequent experiments of Gortner, Lawrence and Harris (1916) have confirmed these conclusions.

For the determination of the osmotic pressure of the expressed liquid the method usually employed is that depending on the lowering of the freezing point of the solution. The thermo-electric method of determining the freezing point lowering evolved by Dixon (1911) may be mentioned in this connection. Harris and Gortner (1914) have published a useful table giving the values of the osmotic pressure for depressions of the freezing point between 0.001° and 2.999° C.

This method, of course, gives the mean osmotic pressure of all

the cells in a tissue or organ. This may be an advantage or disadvantage according to the end in view¹.

THE MAGNITUDE OF THE OSMOTIC PRESSURE IN PLANT CELLS

A great number of determinations of the osmotic pressures of plant cells and tissues have been made by the plasmolytic method and by determinations of the freezing point of expressed saps. With the vast array of data so obtained it is not proposed to deal in any detail here. Some reference should, however, be made to the magnitude of the osmotic pressure in plant cells, and to the variations in the osmotic pressure related to the ecological type of plant, the habitat of the plant, the position of the organ or tissue, the time of day and season of the year.

Unfortunately different authors have expressed the osmotic pressures of the cells examined in different units. Thus Sutherst simply gives the freezing point lowering, Ursprung and Blum give the concentration of sucrose or potassium nitrate isosmotic with the sap (the "cane sugar value" or "saltpetre value") while Dixon and Atkins and Harris and his collaborators give the pressures in atmospheres and Livingston (1903) in millimetres of mercury.

Pfeffer (1900), basing his remarks on older observations (de Vries, 1884*a*; Stange, 1892; Janse, 1887*a*), states that the osmotic pressure in the cells of land and fresh water plants is usually of the order of 5 to 11 atmospheres, but the recent observations of Dixon and Atkins, Harris and his collaborators, and Ursprung and Blum, clearly show that the normal range of osmotic pressures of plant cells is wider, extending much further in the upward direction. Thus out of 53

¹ The method of tissue tension has not been included among the methods for determining the osmotic pressures of cells for the reason explained in the footnote on p. 180; that is, on account of the presence of turgor pressure, any results obtained with turgid cells must necessarily be approximate, and with highly turgid tissues will be exceedingly incorrect. The same holds with regard to the change-in-weight (or change-in-volume) method. But for cells without an enveloping elastic envelope like the cell wall, a liquid in which cells neither lose nor gain in weight, that is, in which no interchange of water takes place, is to be regarded as isosmotic with the cell sap. The method is thus applicable to the determination of the osmotic pressure of some animal cells (H. J. Hamburger, 1886, 1887, 1889, 1890; Gryns, 1896; Köppe, 1895, 1897; Löb, 1894; Hedin, 1895 *a*, 1895 *b*, 1896), and also to a few plant cells without cell walls.

By attaching weights to tissue, rendered turgorless by plasmolysis or death, until the tissue has regained its original length, it is possible, according to Pfeffer (1903), to calculate approximately in some cases the original osmotic pressure of the tissue. As such a method can only be very approximate at best, no further mention of it need be made here.

determinations tabulated by Dixon and Atkins (1913 *b*) the osmotic pressure was less than 11 atmospheres in only 13 cases, the lowest value found being 5.83 atmospheres in the leaf of *Saccharum officinarum* and the highest 38.32 atmospheres in the fruit of *Vitis vinifera*. According to Pfeffer (1900) the osmotic pressure, even in starved cells, rarely falls below 3.5 atmospheres (Stange, 1892; Copeland, 1896), while at the other end of the range saprophytic fungi, such as *Aspergillus niger* and *Penicillium glaucum*, growing on concentrated nutrient solutions, may develop an osmotic pressure of the sap of as much as 157 atmospheres (Eschenhagen, 1889). It is clear that in such cases the suction pressure is very great when the cells are transferred from the concentrated medium in which they were growing to distilled water, and it is not surprising that under such circumstances the cell walls may be ruptured (Pfeffer, 1900). The highest value recorded for the osmotic pressure of the sap of a flowering plant appears to be 153.1 atmospheres found by Harris, Gortner, Hofman and Valentine (1921) for a plant of *Atriplex confertifolia* growing in the neighbourhood of the Great Salt Lake.

THE RELATION BETWEEN THE POSITION OF A CELL IN THE PLANT AND THE OSMOTIC PRESSURE OF THE CELL

This is a question to which some considerable attention has been given, chiefly in relation to the ascent of water in trees. Ewart (1905) made some determinations of the osmotic pressure of leaf cells by the plasmolytic method and thought he had shown that the concentration of the sap of leaf cells increases from lower to higher levels of the plant, but later (1906) came to the conclusion that the errors inherent in the method were too great to allow the drawing of definite conclusions. From the determinations of Dixon and Atkins (1910) it is concluded by Dixon (1914) that on the whole leaves at a lower level contain sap with a lower osmotic pressure than that of leaves inserted at a higher level, but that their results are not altogether satisfactory from this point of view. It is to be noted that these determinations were made before these workers realised the importance of a preliminary freezing of the tissues in order to obtain a real sample of the sap when pressed from the tissues. Hannig (1912), using the plasmolytic method, found the osmotic concentration higher in leaves than in roots. His statement that Dixon (1910) found the osmotic concentration of leaves independent of the level of their insertion is scarcely an exact statement of this author's results. Harris, Gortner and Lawrence (1917) made 26 sets of determinations, in-

volving material from 12 species of trees, of the osmotic pressures of expressed saps from leaves inserted at different levels, and found almost without exception that the osmotic pressure is higher in leaf cells the higher the insertion of the leaf. Some of their results are shown in the following table. The slight exception recorded in the table in the case of *Betula lutea* might easily be due to experimental error.

TABLE XX

Osmotic Pressure of the Sap of Leaves inserted at different Levels. (Data from Harris, Gortner and Lawrence)

Species	Height in feet	Osmotic pressure in atmospheres
<i>Betula lutea</i>	66	15.55
	52	16.01
	39	15.12
	25	14.11
	11	12.63
<i>Quercus Prinus</i>	47	20.23
	36	20.08
	30	19.72
	19	19.57
<i>Robinia Pseudacacia</i>	51	12.44
	39	11.07
	29	10.87
	9	10.68

An extensive series of observations made by Ursprung and Blum (1916 *a*) by means of the plasmolytic method scarcely supports the conclusions of Harris, Gortner and Lawrence, but the species used were not the same; they were: *Helleborus foetidus*, *Urtica dioica*, *Fagus sylvatica*, *Sedum acre* and *Funaria hygrometrica*. Thus in the case of *Fagus sylvatica* Ursprung and Blum could find no connexion between the osmotic pressure of the cells and the height from the ground of the leaves containing them, while generally the cells of the same tissue (root, stem, leaf stalk and lamina) have a higher osmotic pressure the nearer they are to the *base* of the organ. Other conclusions of Ursprung and Blum are that cells of the same layer at the same height from the ground have approximately the same osmotic pressure if they are not too far apart, but neighbouring cells belonging to different layers may differ considerably in their osmotic pressures. In the cells of younger leaves the osmotic pressure was less than in older leaves.

The highest values for the osmotic pressure were found in *Helleborus* and *Urtica* in the palisade. The same was the case with *Fagus*, but here equally high values were found in the palisade, wood

parenchyma and medullary rays. Minimum values were found in the lower epidermis of the leaf in *Helleborus* and *Fagus* and in the cortex of the leaf stalk in *Urtica*. The mean values found by Ursprung and Blum for *Fagus sylvatica* are shown in the following table. The osmotic concentrations are given as concentrations of potassium nitrate.

TABLE XXI
Mean Values of the Osmotic Concentrations in the Cells of
Different Tissues of *Fagus sylvatica*
(Data from Ursprung and Blum)

Organ	Epidermis	Spongy tissue	Palisade	Outer cortex	Inner cortex	Phloem parenchyma	Companion cells	Cambium	Xylem parenchyma	Cortical medullary rays	Xylem medullary rays
Leaf lamina } Branch Stem Root	0.371 — — —	0.571 — — —	1.017 — — —	— 0.667 0.696 0.65	— 0.671 0.696 0.671	— 0.573 0.562 0.583	— 0.721 0.746 0.70	— 0.64 0.638 0.625	— 1.008 0.963 0.979	— 0.808 0.929 —	— 0.954 0.921 —

DIFFERENCES OF OSMOTIC PRESSURE IN SPECIES OF DIFFERENT HABIT

While the numbers quoted in the preceding section are sufficient to show that great variations of osmotic pressure may occur in different cells of the same species, it is certainly true that differences in the osmotic concentration of cells occur in relation to specific differences. Such differences are frequently related to the habit of the plants under consideration. Thus in succulents the osmotic pressures of the cells are relatively lower than in mesophytes (Ursprung and Blum, 1916 *a*). In an investigation on the plants growing in the deserts of Arizona, Harris, Lawrence and Gortner (1916) found considerable variation of osmotic concentration of different species, the different groups in order of increasing concentration being (1) winter annuals, (2) perennial shrubs, (3) dwarf shrubs and half shrubs, and (4) shrubs and trees. In a later investigation on the osmotic concentration of the leaf sap of plants from the Blue Mountains of Jamaica, Harris and Lawrence (1917 *b*) found that the osmotic concentration of the leaves of woody plants was greater than that of leaves of herbaceous plants, and the same result was obtained with plants of the Arizona deserts and the north shore of Long Island (Harris, Gortner and Lawrence, 1921 *a*, 1921 *b*). Ursprung and Blum (1916 *a*), however, found the osmotic concentration of the palisade cells of *Urtica dioica* about the same

(1.015 *M* KNO₃) as that of *Fagus sylvatica* (1.017 *M* KNO₃), while the mean values for epidermis and spongy parenchyma were higher in both *Urtica dioica* and *Helleborus foetidus* than in *Fagus sylvatica*.

That there might be some fundamental relation between the osmotic concentration of the cells of parasitic plants and their hosts was suggested by MacDougal and Cannon (1910) and MacDougal (1911 *a*, 1911 *b*). Since then observations by Senn (1913), and a number of observations by Harris and Lawrence (1916) and Harris and Valentine (1920) on montane rain-forest and desert Loranthaceæ indicate that the osmotic concentration of the cells of the parasite is in general higher than that of the leaves of the host. The values set out in Table XXII, taken from Harris and Valentine, indicate the degree of difference in the osmotic pressures of the cells of host and parasite.

TABLE XXII

Osmotic Pressures of the Cells of Host and Parasite
(Data from Harris and Valentine)

Parasite and host	Osmotic pressure of leaf sap of host in atmospheres	Osmotic pressure of sap of parasite in atmospheres
<i>Phorodendron Californicum</i>		
on <i>Acacia greggii</i>	26.57	33.66
on <i>Olneya tesota</i>	25.24	26.98
<i>Phorodendron Cockerellii</i>		
on <i>Populus wislizeni</i>	22.04	23.05
on <i>Salix wrightii</i>	20.88	24.98
on <i>Fraxinus attenuata</i>	23.47	26.47

Harris (1918) has shown that epiphytes have an abnormally low osmotic pressure in their cells, the osmotic pressure in the epiphytic species examined by him being 37 to 60 per cent. lower than that of the expressed sap of herbaceous forms, and 28 to 45 per cent. lower than that of ligneous forms.

Thus the evidence available appears to indicate that the osmotic pressure of plant fluids depends in part on the ecological type of plant. Harris and Popenhoe (1916) even claim to have shown a slight difference in the osmotic pressures, as indicated by freezing point lowering, of the expressed sap of the leaves of different horticultural types of the avocado (*Persea americana*).

THE INFLUENCE OF EXTERNAL CONDITIONS ON THE OSMOTIC
PRESSURE OF PLANT CELLS

This question is naturally connected with that discussed in the last section, for differences correlated with the ecological type of a plant may possibly be traced back ultimately to differences in the habitat. Thus Harris, Gortner, Hofman and Valentine (1921) found abnormally high osmotic values for the expressed sap of plants growing in the neighbourhood of the Great Salt Lake, the sap in two plants of the salt desert half shrub *Atriplex confertifolia* growing on rocky cliffs possessing osmotic pressures of 82.9 and 94.7 atmospheres respectively while three plants of the same species growing on low ridges in salt flats gave values of 74.2, 118.5 and 153.1 atmospheres respectively. The great difference in the osmotic pressure of the sap of plants of rain forest and of desert, even when the plants are of similar growth forms, may be correlated with differences in the environmental factors. Harris and Lawrence (1917 *b*) found the average osmotic pressure of the sap of ligneous forms in the rain forest of the Blue Mountains of Jamaica was about 11.44 atmospheres as compared with 14.40 for ligneous plants growing in Long Island and 24.97 atmospheres in the case of plants growing in the deserts of Southern Arizona (Harris, Lawrence and Gortner, 1916). Similar differences were found with herbaceous forms, the average osmotic pressure of the sap of Blue Mountain, Long Island and Arizona desert forms being 8.80, 10.41 and 15.15 atmospheres respectively. High values of osmotic pressure in leaves of desert plants have also been observed by Fitting (1911) and Keller (1913).

Iljin, Nazarova and Ostrovskaja (1916) measured the osmotic pressure of cells of the roots and leaves of a number of swamp, meadow and steppe plants by means of the plasmolytic method, and compared the values obtained with the conditions of the surrounding medium, soil or air, as the case might be, as regards water content. In roots they found the highest osmotic pressures in steppe plants, lower values in meadow plants, and lowest values in swamp plants, the values obtained for these three groups of plants being respectively 0.40–0.48, 0.19–0.30 and 0.13–0.20 of the osmotic pressure of a normal solution of sodium chloride. The water content of the soil or swamp was in inverse order, so that it appears that the osmotic pressure of root cells is less in roots growing in a soil with a higher water content. Observations on a number of plants common to steppe and meadow confirmed this. Thus in the cases of *Poa pratensis*, *Triticum repens*, *Kæleria gracilis*, *Stipa capillata*, *Festuca*

ovina, and *Lythrum virgatum*, the osmotic pressure of the cells of the root was always higher in the plants of any one species growing in a drier soil than in roots of plants of the same species growing in a soil of higher water content. Similar relations between the water content of the soil and the osmotic pressure of the cells of root and shoot of maize have been found by McCool and Miller (1917) by the expressed sap method.

Iljin, Nazarova and Ostrovskaja found that the osmotic pressure of the cells of the root is no criterion of the osmotic pressure of the cells of the leaves. They come to a conclusion similar to that in regard to the influence of the water content of the soil on the osmotic pressure of the cells of the root, namely, that the water content of the air surrounding the leaves influences the osmotic pressure of the leaf cells: the higher the water content of the air the lower the osmotic pressure of the leaf cells. Thus leaves of the same plant, for example, *Senecio doria*, *Centaurea scabiosa* and *Sanguisorba officinalis*, had all the same osmotic pressure in their cells when growing in the open and therefore with all the leaves under the same external conditions as regards moisture, but when growing among grass the lower leaves always gave lower values of the osmotic pressure than the upper leaves. Drabble and Lake (1905, 1907) also found by the plasmolytic method higher osmotic pressures in the epidermal cells of leaves of those plants most subjected to factors tending to loss of water by transpiration.

Confirmatory evidence of the influence of water content of the soil on the osmotic pressure of the plant sap is forthcoming from the results of Pringsheim (1906), Meier (1915), Ursprung and Blum (1916 c) and Iljin, Nazarova and Ostrovskaja (1916). Plants of a number of species were grown by these various investigators under different conditions of humidity and the osmotic pressure of the sap of the various experimental plants examined. The result is quite general that higher water content of the environment corresponds with lower osmotic pressure of the cell sap, and *vice versa*. Even as long ago as 1884 de Vries recorded a higher osmotic pressure for shoot apices of *Helianthus tuberosus* after dry weather than after a long period of daily watering.

An increase in the concentration of substances dissolved in an aqueous medium is of course equivalent to a decrease in the water content. From the observations just recorded it is therefore not surprising to find that plants growing in strong solutions of osmotically active substances generally exhibit an increase in the osmotic pressures of their cell sap. Thus Janse (1887 a) showed that the osmotic

concentration of the cell sap of *Chaetomorpha* increased as a result of transferring it to a solution of higher salinity, while Mayenburg (1901) found a similar increase in osmotic pressure in *Aspergillus niger* when this fungus was grown on strong nutrient solutions. The osmotic pressure of the cell sap of root hairs of halophytes appears to be considerably higher than that of mesophytic plants (cf. Drabble and Lake, 1905, 1907), while T. G. Hill (1908) has shown that the osmotic concentration varies with that of the external medium and in the same direction. It is, however, not at all clear whether the increase in osmotic concentration is due to simple absorption of the salt or other osmotically active substance from the external medium, or to a complicated series of internal changes not understood (cf. T. G. Hill, 1919, for halophytes; Mayenburg, 1901, for *Aspergillus*). According to von Faber (1913) the osmotic concentration of the cell sap of mangroves is considerably higher than the normal, but only in some cases is this due to a high concentration of sodium chloride. Stange (1892) could find no accumulation of potassium nitrate in the cells of the root tips of *Pisum* and *Lupinus* growing in strong solutions of this salt although the parenchyma cells behind the root tip contained the salt in considerable quantity.

Wind, no doubt by its influence on transpiration and consequent removal of water from the leaf cells, induces an increase of osmotic pressure in them (Ursprung and Blum, 1916 c).

The data bearing on the influence of temperature on the osmotic pressure of plant cells have been brought together by Ursprung and Blum (1916 c; see also Blum, 1917). From their own observations and the earlier ones of Copeland (1896), Pantanelli (1904), Lidforss (1907), Kny (1909), Winkler (1913), Buchheim (1915) and Meier (1915) it would appear that the osmotic pressure of the cells examined is a minimum in the region of 10° to 12° C. and increases with both increase and decrease in temperature. Yet the influence of temperature has not been wholly disentangled from the influence of other factors, and it would be premature to accept such an opinion as conclusively proved.

With regard to the influence of light on the osmotic pressure of plant cells, de Vries (1884 a) and Copeland (1896) have shown that etiolated plants give lower values of the osmotic pressure in their cells than normal plants. Buchheim (1915) concluded from observations on the alga *Cylindrocystis* that strong illumination brought about an increase in the osmotic pressure of the cell sap of this plant. Dixon (1914) found that illumination increased the osmotic pressure

in leaf cells and attributes this, no doubt correctly, to the formation of carbohydrates. Meier (1915) found in a number of species a somewhat higher osmotic pressure in the leaves exposed to the sun than in leaves of the same species growing in shady habitats. Observations by Ursprung and Blum (1916 c) on *Funaria* showed the same relation, while the same authors found higher osmotic pressures in epidermal cells and most of the mesophyll cells of sun leaves of the beech as compared with the corresponding values found in shade leaves of the same species. Intense illumination thus appears to lead to an increase in the osmotic pressure of leaf cells, as is indeed generally supposed.

PERIODICITY IN THE VALUES OF OSMOTIC PRESSURE IN PLANTS

The various factors of the environment undoubtedly influencing the osmotic pressure of plant cells, it is only to be expected that in a climate with a daily and annual periodicity there should be a corresponding periodicity in the value of the osmotic pressure of the cell liquids. A daily periodicity in the value of the osmotic pressure of the cells of the different tissues and in different parts of the plant has been shown by Ursprung and Blum (1916 b) in *Helleborus foetidus*, *Fagus sylvatica* and *Sedum acre* and also in the leaves of *Funaria*, the osmotic pressure increasing from early morning until the afternoon and then falling until early the next morning. This course runs parallel with the temperature and inversely as the humidity.

An annual periodicity has also been made out by the same authors, their results indicating on the whole minimum values in the summer and maximum values in the winter. There are, however, many departures from this, as would be expected when one considers that the changes in the various environmental factors are by no means regular.

THE SUBSTANCES IN THE PLANT RESPONSIBLE FOR THE OSMOTIC PRESSURE

It has been noted in Chapter VI that an osmotic pressure can only be developed when two solutions are separated by a membrane which is impermeable, or only little permeable, to solute or solutes on one side of the membrane. On the simple osmotic view of the plant cell we have therefore to suppose that in the vacuole there are present solutes to which the protoplasm is impermeable, and that these solutes are in such concentration as to give rise to the high osmotic pressures noted in previous sections of this chapter. To determine the substances responsible for the osmotic pressure de Vries

(1884 *a*) analysed the expressed sap from a number of plant organs and calculated the osmotic pressure due to each of the substances present. He found the sum of the osmotic pressures so obtained approximately equal to the osmotic pressure of the expressed sap as determined by the plasmolytic method. A number of his results are summarised in Table XXIII.

TABLE XXIII
Osmotically active Constituents of Cell Sap
(Data from de Vries)

Constituent	Osmotic pressures (as saltpetre values) of the various constituents in different species						
	<i>Heracleum sphondylium</i> Leaf Stalk	<i>Gunnera scabra</i> Leaf stalk		<i>Rheum officinale</i> Stem	<i>Rheum hybridum</i> Leaf Stalk	<i>Rochea falcata</i> Leaf	<i>Rosa hybrida</i> Petal
		Young	Old				
Organic acids	0·020 ¹	0·023 ¹	0·028 ¹	0·063 ²	0·124 ²	0·055 ¹	0·023 ³
Potassium salts of organic acids	0·013	0·004	0·004	0·012	0·013	0·004	0·012
Glucose	0·152	0·026	0·021	0·085	0·052	0·030	0·218
Potassium chloride	—	0·062	0·090	—	—	—	—
Sodium chloride	0·014	—	—	—	—	0·015	—
Potassium phosphate	—	—	0·003	0·012	0·007	—	—
Total	0·199	0·115	0·146	0·172	0·196	0·104	0·253
Found saltpetre value of sap	0·22	0·12	0·16	0·20	0·22	0·13	0·27

¹ Principally malic acid and calculated as such.

² Principally oxalic acid and calculated as such.

³ Not identified with certainty but probably malic acid and calculated as such.

These results and others show that the osmotically active substances of the cell sap are very varied. De Vries (1879, 1883) had previously emphasised the importance of organic acids in the maintenance of turgor, but his later work showed that this is by no means a general rule. Nevertheless, in *Rheum hybridum* oxalic acid, and in *Rochea falcata* malic acid, play a leading part in maintaining the osmotic pressure of the cell sap, while the observations of Kraus (1886) on a number of Crassulaceæ show that in these plants half the dry weight may consist of soluble salts of malic acid. High percentages of oxalic acid in the sap were found by de Vries in the cases of leaf stalks of *Begonia Rex* and *B. manicata*, in which free oxalic acid and potassium oxalate accounted together for about the osmotic pressure of the sap.

The percentage of the osmotic pressure of cell sap due to sugars was found by de Vries to be very varied. The sugar is always calculated as glucose by de Vries, but it will probably be more accurate to regard his numbers for glucose as approximately those for total sugar,

although of course where sucrose is present the osmotic pressures calculated on the assumption that the whole of the sugar is glucose are too high as the osmotic pressures of equal weights of sucrose and glucose are in the ratio of approximately 1 : 2. As free acids were present in the sap and as the method of analysis of de Vries involved heating the sap it is probable that any sucrose would be inverted and so appear as hexose reducing sugar. The highest values for glucose were found in the petals of *Rosa* and the leaf stalks of *Heracleum sphondylium*, in which the percentage of the osmotic pressure attributed to glucose was found to be 80.7 and 69.1 per cent. respectively. At the other extreme come leaves of potato in which the part of the osmotic pressure of the cell sap attributable to sugar was found to be less than 4 per cent. Between these two extremes all possible intermediate conditions can be found.

Potassium chloride is responsible for more than half the osmotic pressure in the cells of leaf stalks of *Gunnera scabra*, while in other plants other inorganic salts may form an important fraction of the total osmotically active substances, as for instance in growing shoot apices of *Helianthus tuberosus*, where de Vries found potassium nitrate responsible for 41.4 per cent. of the total osmotic pressure. Copeland (1896) concluded that the same salt was mainly responsible for the osmotic activity in *Fagopyrum*, *Pisum*, *Phaseolus* and *Zea*. The work of Nathansohn (1901) on *Codium tomentosum* suggests that in this plant sodium chloride may be an active osmotic constituent of the cell sap, while the more recent work of Wodehouse (1917) indicates the presence of a number of inorganic salts in the sap of the marine alga *Valonia*. It is probable that in marine plants generally, and also in halophytes, sodium chloride particularly may play a prominent part in the maintenance of osmotic pressure of the sap. That such is indeed the case cannot, however, be accepted without question (cf. T. G. Hill, 1919). Von Faber (1913) states that in mangroves the high osmotic pressures are due to sodium chloride in some species but not in others.

It will be noted that a dissolved substance in the cell sap can only be a factor in the maintenance of a permanent osmotic pressure if the protoplasm is for all practical purposes impermeable to the substance. Now it is to be expected from the general considerations in earlier chapters that substances of small molecular weight such as potassium chloride and potassium nitrate and other inorganic salts would penetrate the protoplasm with comparative ease and rapidity as they do through membranes artificially prepared. There

is undoubtedly a very real difficulty here to which it will be necessary to return later. It is an attractive theory to suppose that whereas the protoplasm is readily permeable to inorganic salts, it is impermeable to organic substances with larger molecules such as carbohydrates and organic acids, and that the osmotic pressure of the cell sap is to be attributed to these. This will, however, not explain the results of de Vries and Copeland recorded above.

Calculations of the molecular weight of the solutes in the expressed sap of seedling peas made by Maquenne (1896) by means of determinations of the freezing point, suggest an average molecular weight of 239, considerably higher than that of glucose, namely 180. On the other hand, the molecular weight of the solutes in the sap of *Helianthus* was found to be on the average only 136, and as glucose was present in the sap, it would appear that the osmotic pressure must be ascribed in large part to substances with lower molecular weights than that of glucose. Rather similar values were obtained by Dixon (1914) for the mean molecular weight of the solutes in the leaf sap of *Wistaria sinensis* and *Ulmus campestris*, the values in the former varying from 149 to 169, and in the latter from 148 to 165.

Comparison of the electrical conductivity of saps, which gives an approximate measure of the content of electrolytes, with the osmotic pressure, shows that there is no constant relation in different species between the content of electrolytes and total osmotically active solutes (Harris, Gortner and Lawrence, 1920, 1921 *a*, 1921 *b*).

THE SUCTION PRESSURE

Although the amount of water which a cell is capable of absorbing must depend on the osmotic concentration of the cell sap and the impermeability of the protoplasmic membrane, it is suction pressure which gives a measure of the pressure with which water is absorbed by the cell. Attention has recently been directed to the suction pressure in plant cells by Ursprung and Blum, who describe methods for determining the full suction pressure (1916 *d*) and who have published the values they have determined for it in a number of cases.

DETERMINATION OF THE FULL SUCTION PRESSURE

Ursprung and Blum (1916 *d*) describe two methods for the determination of the full suction pressure. These may be described as the indirect plasmolytic method and the equilibrium method.

Indirect plasmolytic method. It will be recalled that the full suction pressure equals the osmotic pressure of the cell sap less the wall

pressure. The indirect plasmolytic method consists in (1) measuring the osmotic pressure of the cell by plasmolysis as already described, and (2) in estimating the wall pressure. This is done by assuming that a linear relationship holds between wall pressure and cell volume between the extreme limits when the wall pressure is zero at plasmolysis and when the wall pressure is a maximum and equal to the osmotic pressure of the cell sap, when the cell is in equilibrium with pure water. It has already been pointed out that such an assumption can only be regarded as an approximation.

This method, it will be observed, involves the measurement of the original volume of the cell, its volume at plasmolysis, and its volume when fully swelled in distilled water. It is also necessary to determine the osmotic concentration of the cell.

Equilibrium method. The principle of this method was clearly enunciated by de Vries (1884 *a*) in reference to the determination of the suction pressure of transpiring shoots, but there are apparently no records of any measurements made by him. It consists simply in determining that concentration of a solution of a substance to which the protoplast is impermeable in which the cell undergoes no change in volume. In this case the net suction pressure is zero, so that if P_e is the osmotic pressure of the external solution, P the osmotic pressure of the cell sap and T the wall pressure,

$$0 = P - P_e - T,$$

$$\text{or} \quad P_e = P - T \quad \text{.....(1).}$$

When the cell is placed in distilled water, if S is the full suction pressure which is then exerted,

$$S = P - T \quad \text{.....(2),}$$

so that, as T is the same in equations (1) and (2),

$$S = P_e.$$

Hence the full suction pressure of a cell is equal to the osmotic pressure of a solution of a non-penetrating substance in which the cell undergoes no change in volume. When the suction pressure of tissues is in question it will generally be found simpler to use weight as a criterion instead of volume.

Whichever method of determination is used solutions of sucrose are likely to be the most generally useful external media. It is also to be noted that the method neglects any external forces which may be operative such as tissue tensions. If there should be acting on a cell in the intact organ an external pressure of the same order of magnitude as the suction pressure found for the cell isolated from

the tissues when the external pressure is no longer acting, it is clear that the suction pressure so found gives no measure of the suction pressure of the cell in the uninjured organ. It is therefore necessary to confine the determinations to cells which are under no appreciable external pressure in the intact plant, or else to take account of such pressure.

THE MAGNITUDE AND RANGE OF SUCTION PRESSURES IN PLANTS

Ursprung and Blum (1916 *e*) have investigated the range of suction pressures in roots and shade leaves of *Fagus sylvatica*. Their chief results are as follows.

In all the leaves examined Ursprung and Blum find differences in the full suction pressures of the cells of different tissues, the order being under epidermis, upper epidermis, guard cells, spongy tissue, palisade. In all these tissues the suction pressure was found to increase with increasing distance from the ground. The values found for root apices 40 cms. below the level of the ground were the lowest of all, but root apices 10 cms. below ground gave abnormally high values. Apart from these last, which are completely unexplained, the mean values given by Ursprung and Blum's determinations are summarised in Table XXIV.

TABLE XXIV
Full Suction Pressures in Cells of *Fagus sylvatica*
(Data from Ursprung and Blum)

Distance from root apex in metres	Suction pressures in atmospheres					
	Root apex	Upper epidermis	Under epidermis	Guard cells	Spongy paren- chyma	Palisade
0.0	5.3	—	—	—	—	—
2.7	—	5.9	7.5	8.1	11.1	15.0
8.7	—	8.4	9.3	9.9	12.4	15.6
11.1	—	9.3	9.9	9.9	14.3	17.1
13.0	—	9.9	10.5	10.5	14.3	17.1

There appears to be here definite evidence of a gradient in the suction pressure of cells of the beech in proceeding from lower to higher levels. This is obviously of the greatest importance with regard to the questions of transpiration and the ascent of water in trees, questions which, however, cannot be discussed in this place.

Further observations of Ursprung and Blum show that after rain the suction pressure is considerably reduced, as, for instance, in one observed case of palisade cells, from about 15.6 to 11.1 atmospheres. Also, as with osmotic concentration, there appears to be a daily periodicity in the values of the full suction pressure, there being in general a minimum in the early morning and a maximum in the afternoon.

In later papers Ursprung and Blum (1918 *a*, 1918 *b*, 1919) have extended their observations to other plants, and have in general confirmed the results of their earlier work. Thus in ivy they found that the suction pressure increased with distance from the absorbing zone of the root in any particular tissue, while in a cross-section through an organ the suction pressure increased with distance from the water conducting tissue. The only exception to this rule was found in the absorbing zone of the root, in which, as might be expected, the suction pressure is greater in the cortex than in the piliferous layer.

In their most recent paper (1921) these same authors pay particular attention to the absorbing zone of the root in *Phaseolus vulgaris* and *Vicia Faba*. They find that the suction pressure increases from the piliferous layer inwards as far as the innermost layer of the cortex outside the endodermis, but that the suction pressure of the latter is considerably less than that of the cortical cells outside it, and higher than that of the pericycle within. They regard this anomaly as most probably explained by the presence of different suction pressures at different points on the surfaces of the cells of the endodermis (cf. the last section of this chapter). The suction pressure as measured is the mean suction pressure in cases where the suction pressure varies over the surface of the cell, and if the suction pressure on the outer side of the endodermal cells should be higher than that of the neighbouring cortical cells, and on the inner side lower than that of the adjoining pericycle cells, the entry of water into the central cylinder can be explained.

As regards the value of the suction pressure in other cases, Stiles and Jørgensen (1917 *b*) have shown that a sample of potato tuber neither gained nor lost weight in a $M/4$ solution of sucrose, which indicates a full suction pressure of about 6.5 atmospheres. An experiment with carrot root indicated a suction pressure of about 17 atmospheres. The suction pressure in these cases depends obviously on the previous history of the experimental material, and especially on the degree of desiccation.

EXUDATION AND ROOT PRESSURE

The exudation of liquid water or a solution from superficial cells of a plant on to the outside surface of the cells takes place in certain cases, as in water pores (water stomata) and nectaries. What appears to be an essentially similar thing is met with in the bleeding of cut plants and in the phenomenon of root pressure, except that in the latter exudation takes place into dead xylem elements instead of on to the surface of the plant.

Several explanations of this exudation of liquid from plant cells have been offered by Pfeffer and subsequent workers, but what is the true explanation must be regarded as still remaining in doubt.

The cells immediately concerned are the exuding cells themselves with an osmotic concentration C_1 corresponding to an osmotic pressure P_1 , and the cells internal to, and bordering on, these with an osmotic concentration C_2 corresponding to an osmotic pressure P_2 . The suction pressures of the exuding and neighbouring cells respectively are S_1 and S_2 . Then if $S_1 > S_2$ water is absorbed from the neighbouring cells. This will proceed until the increased turgor of the excreting cells reduces S_1 until water is no longer absorbed. But if the outer wall of a superficial cell is permeable to water, water will continually pass through this wall on account of evaporation if for no other reason, so that S_1 will be continually increased and there will be in consequence a continual passage of water into the excreting cells from the neighbouring ones. The difficulty is not to account for the movement of water, but for a movement of water sufficiently rapid to bring about an exudation of liquid.

One theory of the mechanism of the exudation of water supposes the protoplasm is more permeable to dissolved substances on the side towards the pore (or xylem vessel) than on the side bordering on neighbouring cells. This was supposed to be the case by Lepeschkin (1906) in the exudation of water by the hydathodes of *Phaseolus multiflorus*, and has been applied by Priestley (1920) to explain the existence of root pressure. It is not proposed to deal here in detail with Lepeschkin's theory of water excretion, for it involves fallacies which have already been noted in these pages and in other writings; it may, however, be worth while to consider briefly the theory of differential permeability of the different parts of the protoplasm (or plasma-membrane) of exuding cells. Let us suppose we have the extreme case in which the protoplasmic membrane is completely impermeable to solutes on the side of the neighbouring cells, but is

somewhat permeable to solutes on the side towards the pore or xylem vessel. Using the same nomenclature as before and assuming the suction pressure is uniform over the whole surface of a cell, if $S_1 > S_2$ water will enter the exuding cells and will only cease to do so when $S_1 = S_2$. But in the meantime solutes are diffusing out from the exuding cells into the pore and the liquid in the pore will thus develop a suction pressure of S_0 equal to the osmotic pressure P_0 , since the turgor pressure is zero. This suction pressure will continue to increase until no further exosmosis of osmotically active solutes takes place from the exuding cells, while at the same time the suction pressure of the exuding cells is decreasing. A time will therefore arrive when $S_0 (= P_0)$ increases to a value greater than $S_1 (= P_1 - T_1)$, at which point exudation of water will take place. If this is the mechanism of water exudation the conditions for this to take place are thus $P_0 > (P_1 - T_1) > (P_2 - T_2)$.

One explanation offered by Pfeffer was to suppose that the exuding cell possessed a higher osmotic concentration in that part of it bordering on the neighbouring cells than in the part adjacent to the water pore (or water conducting element). The suction pressure will thus be different in different parts of the cell. We can then understand that water might be absorbed by the exuding cell both from the neighbouring cell and the pore. This will result in increased turgor of the exuding cell and consequently the wall pressure will soon balance the osmotic pressure on the side of the pore where the osmotic concentration is low, and absorption of water from the pore will stop. Owing to the higher suction pressure on the side of the neighbouring cell, however, water will continue to enter the exuding cell on that side, the turgor pressure will be further increased and in consequence water will be forced out from the exuding cells into the pore or xylem vessel. As V. H. Blackman (1921) rightly points out, if this process is to continue, there must be some mechanism in the cell to prevent mixing of the solutes in the exuding cells and to maintain the difference in concentration in different parts of the cell.

Ursprung and Blum (1921), as already mentioned, consider that a non-homogeneous suction pressure is exerted over the surface of endodermal cells in the absorbing zone of the root, and they suppose that the same may be the case with the parenchymatous cells bordering on the water-conducting elements. They relate the difference in suction pressure in different parts of the same cell rather to differences in the quantity of water imbibed in the cell wall than to differences in osmotic concentration in the cell as suggested by Pfeffer.

INADEQUACY OF THE SIMPLE OSMOTIC VIEW OF THE PLANT
CELL AS REGARDS ITS WATER RELATIONS

It will be observed that on the simple osmotic view of the plant cell it is assumed that the amount of water contained in the cell wall and the protoplasm undergoes no change with varying conditions, and that the only interchange of water takes place between the vacuole and the external medium. And even when the view is slightly modified so that the semi-permeable membrane separating the external medium from the osmotically active cell sap is regarded as a thin limiting layer of the protoplasm instead of the whole of it, changes in the water content of the cell wall are neglected, while it is assumed that the protoplasm and the vacuole form one uniform solution as regards osmotic properties. It is also assumed that the swelling or shrinkage of the vacuole is solely to be accounted for on simple osmotic grounds. It is clear that we have no right to make these assumptions without good evidence, so that these questions deserve some little consideration.

In this connexion the work of F. E. Lloyd cited in Chapter VIII is of particular interest as indicating the possibility of explaining the water relations of certain plant cells without the supposition of a surrounding plasma-membrane. Some recent observations of Lapique (1921) are also significant. This writer found that when filaments of *Cladophora glomerata* and *C. oligoclona* are immersed in acid solutions the cells become plasmolysed while the cellulose wall swells up and loses its sharp delimitation. The changes appear to be independent of the osmotic concentration of the external solution as they are observed with $N/1000$ hydrochloric acid as well as with $N/10$. Although alkalies appear to have no influence on the normal cell, yet they act on a cell treated with acid in the reverse sense to the acid. Lapique concludes from these observations that the acidity or alkalinity of the external medium is of more importance for the intake of water than the osmotic concentration of the medium. He further supposes that the cellulose wall and the protoplasm constitute colloidal systems in which the particles carry different charges, as one is coagulated by acids and the other by alkalies.

WATER RELATIONS OF THE CELL WALL

It is usually assumed that the cell wall is saturated with water and that this being so, the amount of water present in the cell wall is unaffected by conditions within very wide limits. It has, however, been shown earlier that the cell wall is not simple in chemical composition

and that it may even be complicated in structure. On this account it is to be regarded as quite possible that under different conditions of stretching or with variations of other conditions, the amount of water held by the cell wall may vary. That cell walls might vary as regards the amount of water contained in them appears to have been fully recognised by Pfeffer (1903).

Some experiments of Hansteen (1914) appear particularly suggestive in this connexion. Pith from the leaf stalks of turnips was crushed in a porcelain mortar with water, and after standing for several hours, the solid material was washed repeatedly with water and then re-crushed until no intact cells remained. In this way material was prepared which was regarded as consisting entirely of cell wall. A weighed quantity of this product was dispersed in a very fine condition through cold tap water and allowed to settle on filter paper placed on a sieve. The paper was then removed and dried under pressure in a press. In this way membranes were obtained 90 to 100 μ thick, and used with an area of 3×1 cms. Experiments were conducted on the water uptake of these membranes and their behaviour in this respect compared with that of similar membranes from which lipid substances had been removed by treatment with hot alcoholic hydrochloric acid, and also with membranes of parchment paper.

Such membranes were immersed for definite times in solutions of various salts, and the water absorbed measured by the gain in weight. Measurements were also made of the rate of water loss by evaporation from the membranes after removal from the solutions. In Table XXV are shown some of the results obtained by Hansteen-Cranner with regard to the uptake of water by his artificial cell membranes when immersed in various solutions at 15.5° C., 10 c.c. of liquid being used in each case.

TABLE XXV

Uptake of water by artificial cell-wall membranes in different solutions. (Data from Hansteen-Cranner)

Solution	Concentration in gram-mols. per litre	Uptake of water in per- centage of original weight of membrane	Loss of water from soaked membrane by evaporation in air at 18° C. for 10 mins.
CaCl ₂	0.1 <i>M</i>	152.27	77.61
KCl	0.1 <i>M</i>	205.19	44.77
NaCl	0.1 <i>M</i>	222.00	54.05
Water	—	175.00	62.50
CaCl ₂	0.01 <i>M</i>	175.82	73.75
KCl	0.01 <i>M</i>	294.87	59.13
NaCl	0.01 <i>M</i>	333.34	62.73
Water	—	172.41	61.00

These numbers indicate clearly that the artificial cell membranes take up less water from solutions of calcium chloride than from equimolecular solutions of potassium and sodium chlorides. The absorption of water from a 0.2 *M* solution of calcium nitrate was also less than that from an equimolecular solution of potassium nitrate. Conversely, the water taken up by the membrane in calcium salts appears more easily lost by evaporation than that taken up by the membranes immersed in potassium and sodium salts. As Hansteen found exactly the same relation to hold between the uptake of water and loss of water by evaporation by whole plants with their roots in solutions of salts of the same metals, he concludes that living cell walls are responsible for this difference in water uptake from different salts. The numbers given in Table XXVI, compared with those in the previous table, indicate the similarity in the behaviour of whole plants and artificial membranes.

TABLE XXVI

Uptake of water by oat plants growing in culture solutions of different salts. (Data from Hansteen-Cranner)

Solution	Relative water uptake per gm. of roots	Relative water loss per gm. of transpiring part
<i>N</i> /100 KNO_3	100	100
<i>N</i> /100 NaNO_3	86.34	100.24
0.177 % $\text{Ca}(\text{NO}_3)_2$ (isosmotic with <i>N</i> /100 KNO_3)	82.01	100.90
<i>N</i> /100 KNO_3	100	100
<i>N</i> /100 NaNO_3	82.48	77.64
<i>N</i> /100 $\text{Ca}(\text{NO}_3)_2$	76.43	103.91
0.177 % $\text{Ca}(\text{NO}_3)_2$	82.05	113.89

It is to be noted that parchment paper membranes do not behave in the same way as the artificial cell wall membranes, for the uptake of water by parchment paper was almost the same whatever the salt dissolved in the water. Obviously parchment paper membranes cannot be compared physiologically with cell walls. On the other hand, lipoid-free artificial cell wall membranes behave in a similar fashion to the lipoid-containing membranes as regards uptake of water from solutions, even absorbing more water than the untreated membranes, while the parchment paper membranes take up considerably less.

These results suggest strongly that the uptake of water by the cell wall is influenced by the nature of the solutes in the external medium, so that the cell wall cannot be neglected in a complete view

of the water relations of the plant cell. Of course, where the volume of the cell wall is very small in comparison with that of the vacuole the differences in the absorption of water by the cell wall under different conditions may be negligible; in other cases the cell wall must obviously be taken into account.

WATER RELATIONS OF THE PROTOPLASM

Just as the cell wall is capable of absorbing water by imbibition, so is the protoplasm. Indeed, Pfeffer (1900) states that "the cell-wall, protoplasm, and indeed all organized structures have the power of imbibing water and swelling." Experiments with non-living systems such as gelatine, agar-agar, proteins and mixtures of these and other substances, strongly suggest that the capacity of the protoplasm for absorbing water is highly dependent on the concentration and nature of the solution with which it is in contact. Acids and alkalies appear to exert the greatest influence on the power of such systems to absorb water, both increasing the swelling capacity considerably. Very comparable results have been obtained by Loeb (1897, 1898), Fischer (1910) and Miss D. J. Lloyd (1916) with animal tissues such as frog muscle and sheep's eyes. Whether such phenomena are capable of explaining completely the water relations of the non-vacuolate cell without the invocation of a limiting semi-permeable membrane to the protoplasm is a question which has been discussed earlier without our being able to come to any definite conclusion. Further evidence from experiments with plants is badly needed. But that protoplasm has such powers of swelling there can be no doubt, nor can it be doubted that the degree of swelling will be influenced by the solution exterior to it. Substances may also be present in the vacuole to which similar considerations apply. Where the volume of the protoplasm is very small in comparison with that of the vacuole, and where the swelling capacity also is not great, such swelling of the protoplasm can be neglected in comparison with the water exchange due to the simple osmotic relations of the cell. In other cases the swelling of the protoplasm may be an important factor in determining the water relations of the plant cell. This appears to be the case, for instance, in many succulent plants such as the cacti, in which the presence in the cell of pentosan mucilages may render the swelling of the cell contents the determining factor in the passage of water between the cell and the exterior. Similar swelling occurs in red algæ (McGee, 1918).

THE EFFECT OF PERMEABILITY OF THE PROTOPLASM TO SOLUTES
ON THE WATER RELATIONS OF THE CELL

In considering the water relations of the cell on the simple osmotic view it has been assumed that the protoplasm is impermeable to the solutes contained in the cell sap, and also to the solutes present in the external liquid. It is further assumed that the external medium exerts no influence on the permeability of the protoplasm. Where these assumptions do not hold our considerations have to be modified.

In the first case let it be supposed that a proportion of the solutes in the cell sap are capable of passing through the protoplasm. These solutes will then diffuse through the protoplasm until the concentrations of each particular solute within and without the protoplasmic membrane are in equilibrium with one another. This will mean that the osmotic concentration of the cell sap is probably lessened so that in consequence the cell would take up less water, or lose more, than it would if the protoplasm were completely impermeable to all the solutes in the vacuole. On the other hand, if there are substances in the liquid external to the cell which can penetrate the protoplasm, the cell, when equilibrium is reached, will contain more water than it otherwise would, since the osmotic concentration is raised. Probably in most cases both phenomena occur, although the former may take place to a very limited extent. Some substances, such as ethyl alcohol, ether, acetone and chloral hydrate, may penetrate the protoplasm so rapidly that plasmolysis is not produced by hypertonic solutions (Overton, 1895). Should the substance external to the cell influence the permeability of the protoplasm, as, for instance, by adsorption or chemical action, so that less or more solute passes through the protoplasmic membrane than otherwise would, the water relations of the cell will be correspondingly altered.

THE EFFECT OF IMPERFECT PERMEABILITY OF THE CELL WALL
TO SOLUTES ON THE WATER RELATIONS OF THE CELL

It is generally assumed on the simple osmotic view of the cell that the cellulose cell wall is completely permeable to most dissolved substances, at any rate, such crystalloidal substances as acids, alkalies, salts and sugars. Apart from the well-known cases of semi-permeable cell walls which have been dealt with in Chapter VII, it is by no means certain that cellulose walls are always as permeable to dissolved substances as to water. Indications of an imperfect permeability of the cell walls for sugar molecules were observed by Wachter (1905) in beet root and onion bulb scales and by

Miss Delf (1916) in onion leaves. How general such a complication may be there is no evidence to tell; it is a possibility which should not be lost sight of in dealing with problems connected with the relations of the cell to solutions.

Where such a complication is present the course of plasmolytic shrinkage will obviously differ from the normal case where the cell wall is as completely permeable to the solute as to water. The rate of separation of the protoplast from the cell wall will take place more slowly, as it will take time for the solution outside the protoplast to reach the plasmolysing concentration.

THE EFFECT OF TISSUE TENSIONS ON THE WATER RELATIONS OF CELLS ENCLOSED IN TISSUES

That different cells in the same organ may be subjected to different external pressures from surrounding cells and tissues is indicated by the changes in shape which pieces of tissue may undergo when isolated from the intact plant body. Thus the curvatures of longitudinal strips of dandelion scapes mentioned in an earlier section of this chapter indicate that in the intact scape the central tissue must have been in a state of compression, for it elongates on isolation of the strip, while the epidermal tissue contracts, showing that this tissue was stretched and under tension. Investigations on tissue strains have been numerous, but a discussion of this subject does not lie within the scope of this work. For general information on the subject the reader is referred to Chapter V of the second volume of the English edition of Pfeffer's *Physiology of Plants* (1903). But the presence of these strains must influence very materially the water relations of the cells in tissues where such strains exist. Yet as far as I know this question is only discussed in one place, namely, in the excellent paper by Höfler (1920) on the osmotic relations of the plant cell. Höfler says himself that the subject has never been investigated either theoretically or experimentally. The relations are obviously complex. Let us consider a tissue which has been immersed in a hypertonic solution, or which has lost water by evaporation, so that all the cells within it are turgorless. If such a tissue is now immersed in water, the latter enters at first on all sides so that neighbouring cells as they swell press against one another. The only space then into which the cells can stretch further is the intercellular space system. Hence the cells become deformed with a non-uniform stretching of the cell wall, and consequently an uneven distribution of the wall pressure. A state of equilibrium is still possible under

these conditions because the pressure balancing the hydrostatic pressure of the cell, the turgor pressure, which must be uniform throughout, is made up of the sum of the wall pressure and a component of the pressure exerted by neighbouring cells, which may vary at different points on the wall of the cell under consideration, since a number of cells are concerned, namely, all the neighbouring cells. Höfler supposes that under these conditions water saturation is reached sooner than it would be in the isolated cell because in addition to the inwardly directed pressure of the stretched wall of the cell itself there is the pressure exerted by the neighbouring cells to prevent further increase of the volume of the cell. If this view is correct, Höfler points out a very significant consequence of it in reference to the suction pressure. As with progressive intake of water the turgor pressure must increase to its maximum more rapidly than it would if isolated, it follows that the suction pressure must increase more rapidly with decreasing turgidity in the case of the cell in a tissue than in the isolated cell, other things being equal, since the suction pressure is equal to the osmotic pressure less the turgor pressure. That is, the suction pressure can vary greatly within a small range of turgidity. This may be of great importance from an ecological point of view.

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

IV. THE WATER RELATIONS OF THE PLANT GROWING POINT

By J. H. PRIESTLEY AND R. M. TUPPER-CAREY

CONTENTS

	PAGE
Introduction	210
I. Experimental Investigation of the Permeability of the Root Apex	211
(1) Experiments with Water under Pressures	211
(2) Experiments with Dyes and Salts in Solution	212
(3) Conductivity Experiments	213
II. A Comparison of the Water Relations of Root and Stem Apices .	215
(1) The Relations of a Meristematic Tissue to Water	215
(2) The Permeability of the Stem Apex	217
(3) Composition of the Wall at the Apical Meristem of Stem and Root	219
(4) Morphological Consequences of the Different Permeabilities of the Apical Meristems	222
III. The Root Apex as an Absorptive Organ?	225
Summary	227
References	228

INTRODUCTION

A RECENT investigation into the mechanism of exudation pressures exerted by the root (Priestley(20)) revealed the necessity for the presence of a tissue formation, throughout the root cylinder, which would be capable of supporting the pressure of the ascending water column, without allowing serious leakage back to the surface of the root. This necessary lateral supporting tissue has been found in the endodermis (Priestley and North(21)) or in the periderm which replaces the endodermis in many older roots.

The endodermal or periderm layer forms a continuous sheath around the central cylinder except for a relatively short region at the root apex where it is replaced by the meristematic tissue of the growing point.

The present investigation began as an attempt to test experimentally the capacity of this meristematic cap closing the endodermal cylinder to retain water held under pressure within the endodermis. Experiments are first described which supply con-

siderable evidence that this meristematic cap is relatively impermeable to the passage of both water and solutes.

In the next section it is shown that a consideration of the conditions necessary for protoplasmic synthesis in meristematic tissue is *a priori* evidence for the conclusion thus reached experimentally.

These considerations have led to a comparison of the relative permeabilities of the apex of stem and root and this in its turn has involved a preliminary study of the development of the cell wall in the meristematic region. Conclusions as to chemical changes in the cell membrane can only be very tentative in the present state of our knowledge but it can be shown that the experimental evidence provides an interesting commentary on the differences between the two apices.

The conclusion that the growing point of the root is relatively impermeable is in fundamental disagreement with the statements of a recent investigator as to the function of the root apex. A third section is therefore devoted to a critical examination of the experimental evidence brought forward by Coupin to show that the root apex functions as an absorptive organ, and further experimental evidence presented from our own work which supports our alternative and very different explanation of Coupin's results.

I. AN EXPERIMENTAL INVESTIGATION OF THE PERMEABILITY OF THE ROOT APEX

The meristematic cap of tissue closing the endodermal cylinder at the root apex is relatively small in extent and not very accessible to experimental treatment. Evidence has been obtained along three different experimental lines which agrees in suggesting that these meristematic tissues are relatively impermeable either to water or to solutes.

(1) *Experiments with water under pressure*

Similar experiments have already been carried out by de Vries (28) who was concerned in demonstrating the relative impermeability of the endodermis to water. In using this method to investigate the resistance of the meristematic cap of tissue to water it was desirable to use as short a length of root as possible so that the full experimental pressure might be exerted upon the meristem itself.

It is not possible to make watertight connections between india-rubber pressure tubing and the delicate tissue of a growing root. The young roots of germinating broad beans (*Vicia Faba* L.) were

therefore allowed to grow into short lengths of tapering glass tubing. After a day or two the roots had become firmly fixed into the tubes with the apex protruding beyond the narrow end. The roots were then cut off, with the glass tube attached, and watertight connections obtained by wiring the pressure tubing on to the wide end of the glass tube. The other end of the pressure tubing was connected to the short arm of a long J-shaped glass tube, by means of which water could be forced into the root with the aid of a column of mercury in the long arm of the tube. A T-join closed by clip or tap, between the short arm of the J and the pressure tubing attached to the root, permitted the removal of air bubbles that otherwise might have accumulated against the cut surface of the root.

When eosin solution was driven into the root under a pressure of one atmosphere no leakage took place at the root apex, not even when the apex was chloroformed and left attached to the apparatus all night. A fine glass capillary was then driven into the root far enough to penetrate the endodermal cylinder, whereupon the eosin solution mounted rapidly in it. When sections were cut off this root the cells of the meristematic cap were only faintly stained although the dye had stained the other tissues deeply, including the cortex (see p. 213). Similarly, no leakage of a solution of malachite green took place when forced into the roots under a pressure of about two atmospheres, until again the endodermis was pierced by a fine capillary tube. The growing point also remained completely unstained, although it is shown later that the meristematic cells stain rapidly with such basic dyes when they come in contact with them. In this case, no movement of the sap in the vascular strand being possible, the basic dye did not reach the meristem within the period of experiment (from one hour to twenty-four hours on different occasions). Experiments on these lines, then, have confirmed the earlier experimental results of de Vries⁽²⁸⁾ and drawn attention to the fact that the apical meristem as well as the endodermal cylinder contributes to retain the sap when under pressure.

(2) *Experiments with Dyes and Salts in Solution*

Many observations have been made upon the entry into the root of dyes and various inorganic salts and these experiments lend considerable support to the view that the meristematic tissues are impermeable to most solutes though they stain extremely readily with basic dyes, in high, probably toxic, concentration. Acid dyes

will not stain living roots after many days immersion. This is very strikingly shown by roots, of cuttings (*Salix* and *Tradescantia* were used), which have been grown continuously in water. These roots will continue to grow in 0.1 per cent. solution of acid dyes such as acid green, and the new apical growth appears completely unstained. On the other hand, many experiments referred to elsewhere (Priestley (20)) have shown that many such dyes penetrate the cortical tissues of the root behind the growing point, diffusing through the walls of the cells until stopped by the impermeable walls of exodermis or endodermis.

In experiments with electrolytes in which the distribution of the kations can be subsequently examined by characteristic colour reaction, no cases have been noted where the kations could be detected in the meristematic cells, except on occasion where toxic effects had clearly preceded or accompanied their entry. Thus in healthy willow roots (*Salix fragilis* L.), the distribution of potassium followed by Macallum's (16) sodium cobalt-nitrite reaction, and in bean and pea roots the distribution of iron taken up from 1/40,000 *N* ferrous sulphate, showed a sharp restriction to the vacuolated region behind the meristematic apex. Iron salts in more concentrated solution frequently proved toxic and then the iron could be detected within the meristematic tissue, but so long as the roots remained healthy and capable of growth iron salts could not be detected within these cells by any reaction either for free or combined iron (Macallum (15); Jones (9)). This statement is supported by a long series of observations made in this laboratory by Miss Helen Heaps, M.Sc., in an investigation of the path followed by iron after its entry into the root. These observations then suggest that though the protoplasts of the root meristem readily take up the colour group of a basic dye, inorganic salts and dyes in solution cannot penetrate this tissue so long as it is alive.

(3) Conductivity Experiments

The high resistance shown by this apical root meristem to the passage of an electric current, suggests that electrolytes are unable to move freely through it. The resistance was experimentally determined with the aid of the apparatus shown in text-figure 1. The bean root was mounted upon a platinum wire with which contact was made through a mercury lead enclosed in a sealed bent glass tube. The bean root was immersed in a paraffin-vaseline mixture of low melting point so that it could be poured into the vessel at

a temperature which did not damage the root. The tip of the root protruded into a salt solution into which a second platinum electrode dipped. The resistance between the platinum wire and upper platinum electrode was then determined by the normal Kohlrausch method, using the alternating current from the secondary of a small induction coil with a high frequency note, and determining the ratio between the root resistance and a known high resistance by finding the null point on a potentiometer wire when connected with these resistances in the ordinary Wheatstone bridge method.

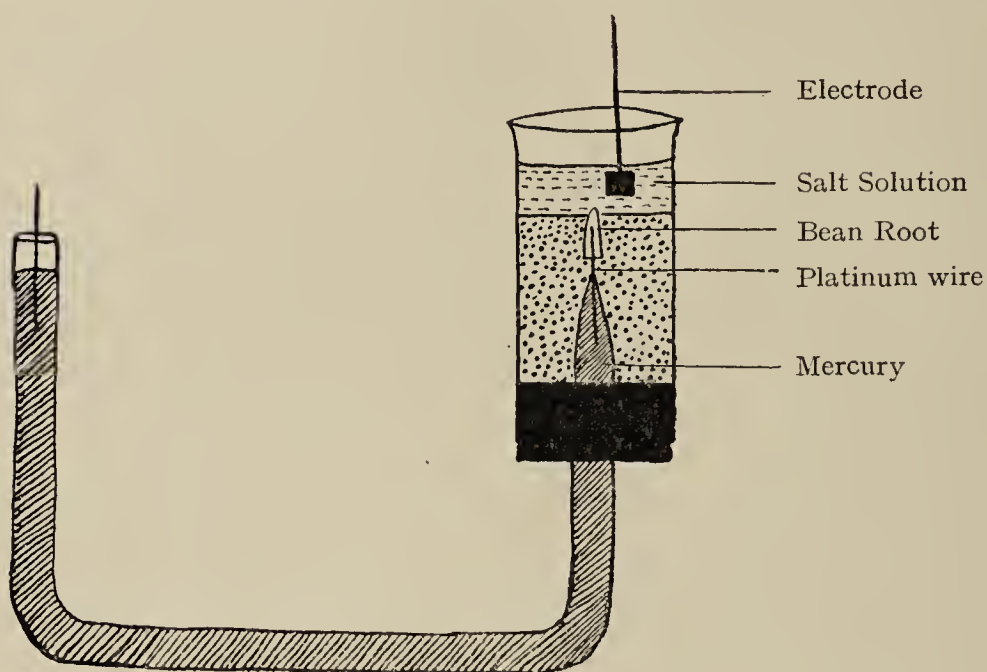


Fig. 1

The roots used were always young root tips of Broad Bean, because their relatively large size permitted their insertion on the platinum wire with the possibility that the wire is entirely within the endodermal cylinder. The resistance of the root tip was then compared with the resistance of the killed root tip and with the resistance of the solution after the root tip has been withdrawn. The resistance of the living roots thus mounted proved very high, from about 2000 to 10,000 ohms in different experiments. After the roots were killed the resistance fell to a few hundred ohms. If root tips were used, taken direct from the dry bean seed and then soaked for an hour in water, the resistance of these roots is still higher, averaging some 12-13 thousand ohms. The reason for this will be apparent later (see p. 221).

At first chloroform was added to the salt solution to kill the root tips, but was extraordinarily slow in action and experience showed that the fall of resistance in the presence of chloroform was little faster than in the normal salt solution alone, as this also proved very toxic. The normal salt solution was then replaced by a balanced salt solution of suitable concentration, the optimal salt solution as ascertained by Shive⁽²⁵⁾ being employed. Whilst in normal salt solution the resistance fell to a few hundred ohms within the first six hours, in this balanced culture solution the resistance remained steady for twenty-four hours, or even increased in amount. Having thus seen that the resistance of the living root remained steadily high, a drop of concentrated sulphuric acid was added with the result that the resistance fell within half an hour in a typical experiment from 7300 ohms to 700 ohms. With chloroform the same effect would be produced in the course of a few hours. Similarly, comparison was made between the resistance of a bean root before and after the meristematic tip was cut away. In this case, also, the removal of the tip was followed by a great increase in the conductivity of the root.

These experiments, then, provide strong evidence that the healthy meristematic tissues are only penetrated with great difficulty if at all by the ions carrying the electric current. In all cases a considerable proportion of this small current that passes will be carried by the surface films around the tissues, so that the actual amount of penetration of the meristematic tissue by the solute present must be very small indeed.

The three lines of experimental evidence thus briefly summarised are concordant in supporting the conclusion that the meristematic tissue at the root apex is relatively resistant to the passage of both water and solutes.

II. A COMPARISON OF THE WATER RELATIONS OF ROOT AND STEM APICES

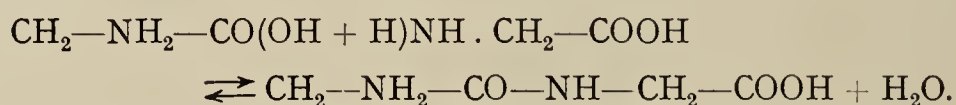
(1) *The Relations of a meristematic tissue to Water*

The following theoretical considerations are now advanced in further support of the experimental evidence that the tissue, closing the endodermis at the root apex, is under normal conditions impermeable to water.

The cytology of any meristem always reveals certain characteristic features. The cells in the apical meristem of the root, for instance, are filled with protoplasm, non-vacuolated and closely

massed together, their thin walls separated by no intervening air spaces. These walls are so thin that even if they were permeable, the passage of water through the tissue would be extremely slow, that is, assuming the protoplasts to be impermeable. But this impermeability of the protoplast, in view of the physiological function of a meristem, seems to be a necessity. Each protoplast forms an active centre of the complex metabolism by which amino-acids and the simpler proteins are converted into that dynamic equilibrium of complex proteins and associated molecules which functions as living protoplasm. We are almost completely ignorant of the chemical nature of the reactions involved in this complex synthesis, but in the light of our knowledge of the hydrolysis of proteins we may expect reversible reactions of the type there met with to be proceeding in the direction of synthesis.

Such a synthesis involves condensation, a chemical synthesis proceeding with the elimination of water, which may be represented in the simplest manner by the following illustration of the condensation of two molecules of glycine to form glycyl-glycine.



There seems no doubt that these reversible reactions, with all possible intermediate stages, play an important part in the protein metabolism associated with protoplasmic synthesis. But unless provision is made for the rapid removal of the products of the reaction, this reaction will soon approach the equilibrium point and proceed no further in the direction of synthesis. Amongst the products of reaction thus requiring removal is water. It is therefore essential that the synthetic reaction should proceed in the absence of water, a condition which is provided for in the closely massed, non-vacuolated and *relatively impermeable* cells of the meristem. The comparatively small amount of water formed during the reaction is probably reabsorbed into the active metabolism of the cell, but its accumulation, or the accumulation of any osmotic substance attracting water into protoplasmic alveolæ, will probably be responsible for the subsequent vacuolation of the meristematic cells as they are left behind the growing point.

That the concentration of water has a material effect upon the direction of such reversible chemical changes as are taking place in the meristematic protoplast is well known and attention is drawn to it by Bayliss⁽¹⁾ (*loc. cit.* p. 239), but the important bearing of the

fact upon the relation of the plant growing point to water has seldom if ever been emphasized.

It must be remembered, however, that this relative impermeability of the protoplast has to be reconciled with the need of the growing tissues for metabolic "Bausteine" from which the protoplasmic complex is constructed. The organic substances required, constituents of the translocation stream, are presumably presented to the surface of the meristematic protoplast in the form of solutes in the cell sap and though the water is unable to enter the cell, the solute must be picked up by the plasma membrane, possibly by a chemical combination succeeding a preliminary adsorption.

These conflicting requirements, coupled with certain fundamental differences in the behaviour of the apices of root and stem to water, suggest possible reasons for the marked morphological differences between the two apices. We must first, however, deal with the experimental work which justifies the statement that the stem apex behaves very differently in its permeability to water and dissolved substances.

(2) *The Permeability of the Stem Apex*

One marked difference between the apex of stem and root is that the meristematic tissue of the stem does not form a cap to the endodermal cylinder. Mylius(19) has shown that the endodermis is never formed so close to the apex in the stem as in the root. In many stems it is never present as a functional layer, with Casparian strip (see Priestley and North(21)). In underground stems and stems growing in water the Casparian strip appears within a relatively short distance of the growing point, but it will be shown in a later paper in this series (Priestley and Ewing(6)) that when the stem apex develops under etiolation conditions the conditions at the apex approximate to those present at the apex of a root. On the other hand, when the stem is developing freely in the light the functional endodermis with Casparian strip is seldom detected in the proximity of the growing point. Mylius(19) points out that a secondary endodermis is frequently present in the aerial stem and that this secondary endodermis when the stem is actively growing can only be found at a considerable distance behind the growing point, but that in the winter its formation appears to overtake the slower activity of the meristem and it can be found very close to the growing point. This we can confirm, but in two cases that have been under very thorough investigation in this department, in

Rubus Idæus L. and *Camellia Japonica* L., it is possible to state quite definitely that when the stem growing point is developing freely in light, no primary stage of the endodermis with typical Casparian strip precedes the formation of the secondary endodermis.

The stem apex is also in possession of a cuticle from its earliest growth in the seedling. Its presence makes it more difficult to investigate experimentally the permeability of the meristem. Stems of *Vicia Faba* L. and *Phaseolus vulgaris* L. were used in these experiments, short lengths bearing apical buds, with all lower nodes cut away to avoid leakage before the pressure reaches the apex. These stem apices were mounted in pressure tubing in the same type of apparatus as in the previous experiments (p. 212) and water, sometimes with various dyes in solution forced into the stem.

The water thus forced into the vessels at the cut end will be unable to make its way out, even if the apical meristem is permeable, so long as the cuticle is unbroken, but directly a small tear is made in the cuticle the liquid oozes out from the surface, if the liquid is under pressure of half an atmosphere or more. The simplest way of demonstrating this fact is to pierce the cuticle with a very fine glass capillary, the liquid then soon rises in the capillary tube. Occasionally it will be found necessary to withdraw the capillary slightly after its first entry as it may get blocked with crushed tissue. These experiments suggested that the meristem at the stem apex was much more permeable to water.

Experiments with dyes in solution in the water forced in, brought to light further differences between stem and root. If acid dyes are used, in which the molecular grouping responsible for the colour behaves as an electro-negative ion, the dye penetrates rapidly the cells at the growing apex and the liquid oozing immediately from any scratch in the cuticle will be coloured with the dye. Even when the cuticle is left intact, the dye finds its way into the apical cells in a few hours. On the contrary, basic dyes, in which the colour group is electro-positive, do not penetrate the apical protoplasts and the liquid oozing from a cut, or driven up an inserted capillary tube, is colourless. In this case the dye is probably held back by the walls of the cells, either by adsorption or chemical reaction: the evidence in favour of the two alternatives will be found in the papers of Wieler⁽²⁹⁾ and Sven-Oden⁽²⁷⁾. Not only, then, is the stem apex relatively permeable, but it stains readily with acid dyes, whilst the root apex is relatively impermeable and only stains with basic dyes.

The relative differences in permeability in these two meristems must be explained by the composition of the intervening walls, the protoplasts in both cases must remain impermeable to water if the arguments advanced in the previous section are sound. The results of a comparison of the walls of these two meristematic tissues are given in the following section.

(3) *Composition of the Wall at the Apical Meristem of Stem and Root*

The most striking difference between the two apices is their behaviour towards the usual reagents for cellulose. The walls of the meristematic cells of the stem apex right up to the layer below the cuticle, treated with iodine in potassium iodide and strong sulphuric acid, give a strong cellulose reaction. On the other hand, when sections of a root apex are similarly treated, the walls of the meristem without exception show no trace of cellulose. The cell walls of the root cap usually give a strong cellulose reaction, as do also the walls of periblem and plerome at varying distances behind the growing point. The plerome remains free from cellulose for a greater distance back than the periblem. This microchemical behaviour of the membrane at the root apex has been previously reported by Solla(26), quoted by Gardiner(7).

The walls frequently are described as of "young cellulose," thus Molisch(18) cites a staining reaction with congo red introduced by Zimmermann as a test for young cellulose in root tips. This reaction with congo red, however, has marked resemblances to the staining behaviour of congo red with proteins such as egg albumin, and has only become known as a reaction for "young cellulose" because it has been assumed that the apical meristem must contain such a substance. Heidenhain, quoted by Mann(17) (*loc. cit.* p. 455), describes congo red, which is the sodium salt of a sulphonic acid, as forming with albumin a congo red albuminate which is transformed into a darker red albumin congo red sulphonate on addition of 10 per cent. acetic acid. The free blue insoluble congo red acid is only liberated from this compound with albumin on addition of a strong mineral acid. When sections of the root apex of *Salix fragilis* (L.), from roots grown in water, were left for twenty-four hours in congo red, the walls of the meristem were stained a deep red which only changed to a darker shade on addition of 10 per cent. acetic acid whilst the stain in the cellulose walls in the older regions of the roots promptly went blue. It was necessary to add a mineral acid before

the meristem walls would release the blue acid. This reaction was extremely clear and the contrast in the behaviour of meristem and older membranes well marked in longitudinal sections of the old root cut so as to pass transversely across the meristem of a young emerging rootlet.

These results suggested that the meristem wall behaved like an amphoteric protein, the isoelectric point of which is on the acid side of the normal sap reaction so that it behaves as an acid when first stained with congo red, but on addition of 10 per cent. acetic acid, in a medium more acid than its isoelectric point, it behaves as a base (see Loeb (10), (11), (12)). This point deserves noting because no cellulose or carbohydrate constituent of the membrane is likely to behave in such a manner; the actual interpretation of the staining reaction of proteins with congo red is still a matter for investigation, it is discussed again by Bayliss (in the 1921 edition of Bolles Lee (p. 125)). This possibly amphoteric behaviour has not been found in other roots growing in damp air, although much attention has been directed to the point, and the behaviour of sections to dyes and various salts carefully studied after previous immersion in a series of buffer solutions of definite P_H value. (See Cole (2) for general principle of these buffer mixtures and Loeb (13) for suggested value of subsequent reaction with silver or nickel salts, etc.)

In all the roots examined the walls of the growing point were more resistant to concentrated sulphuric acid than the normal cellulose wall, dissolving in it very slowly indeed: they also withstood boiling in concentrated potash. In a previous paper (Priestley and North (21)), attention has been drawn to the resistance to concentrated acid and alkali of the apical cells of the stem apex of *Potamogeton perfoliatus* L. In this plant the resistant character is retained by the walls of the layer around the plerome until it differentiates as the endodermis: in the rest of the tissue this character appears only to disappear as the cellulose reaction appears in the membranes. The presence of the resistant substance in a stem apex is attributed to the growth of the plant completely submerged under water, and this substance is thought to be similar to, if not identical with, the substance normally preceding the cellulose membrane at the root apex.

There are reasons for assuming that nitrogen is present in the membrane at the stem apex of *Potamogeton* (Priestley and North (21) p. 123) and therefore the effect of peptic acid and tryptic

digestion upon the membranes of the growing point was investigated. Root tips of *Vicia Faba* L. were digested in acid peptic and alkaline tryptic solution, control root tips being kept in alkaline and acid solution of equivalent strength. All solutions were kept antiseptic with toluol. After several weeks' digestion the tips were fixed in absolute alcohol, embedded in paraffin, microtomed and the sections stained with Delafield's hæmatoxylin and safranin in 70 per cent. alcohol. It could then be seen that while the protoplasts themselves were about equally digested in both cases, the walls of the meristem after peptic digestion had almost disappeared.

In an attempt to isolate the resistant substance present in the root tip, the radicles of *Vicia Faba* L. taken from the dry seed were used, as preliminary tests had shown that sections of these radicles only showed traces of cellulose in the region where they joined the hypocotyl. The dry radicles were ground into a fine powder and then boiled with alcoholic potash to saponify and remove fats. The residue after saponification was soaked in concentrated sulphuric acid for twenty-four hours, then boiled in 10 per cent. acid under a reflux condenser for three hours to remove the cellulose. The residue after filtering, washing and drying was then boiled in 20 per cent. potash, and after being again filtered and washed on a plug of Kahlbaum asbestos in a Gooch crucible, was found to give a strong reaction for cellulose and was now completely soluble in strong sulphuric acid. The only explanation of the result would appear to be that cellulose had been produced as the result of the hydrolysis in potash. Sections of the dry radicles were therefore tested for cellulose after boiling with caustic potash for a moment on the microscope slide when the reaction was much more marked and more widely distributed.

There is very little experimental evidence in the literature available to us of cellulose arising from pre-existing substances, but Czapek⁽¹⁶⁾ (*loc. cit.* p. 644) has pointed out that in the mosses the reaction for cellulose is usually first obtained after boiling with dilute alkali, while it is a general instruction in micro-chemical literature that the cellulose reaction is usually better after such preliminary treatment of the section.

The evidence now available to us in the case of the root tip suggests that in the meristematic region the walls do not give the cellulose reaction but contain possibly more or less protein, together with some substance which increases in amount with time and which gives the cellulose reaction on boiling in potash. In the stem we have not sufficient data as yet about the existing conditions but

the cellulose appears to be formed actually in the meristematic region.

No further relevant information has been obtained as to the nature of the substances that precede cellulose in the membranes in the meristematic region. Sections of the root apex were heated with concentrated caustic potash in sealed tubes in an oil bath to a temperature of 180° C. and subsequently tested for chitosan with 20 per cent. H_2SO_4 and dilute iodine in potassium iodide (van Wisselingh(30)) also with bromine water (Zemplen(31)). A strong reddish-purple coloration was obtained with the dilute iodine when the section was left in the reagent for some hours, the colour approximating very closely to that given immediately by chitosan (or mycosine) prepared from *Polyporus betulinus* and *Psalliota campestris* by Miss L. M. Woffenden according to Gilson's method(8). Comparison with the behaviour of filter paper similarly fused at 180° and then left in the acidified dilute iodine showed that this delayed reaction was given by the cellulose wall and could not be regarded as any indication of the presence of chitosan, but was probably due to the presence of cellulose in the meristematic region as the result of the previous treatment. This reaction, therefore, supplies no evidence for the existence of chitin in the wall prior to its fusion with alkali.

Though we are still without evidence as to the exact nature of the cell wall at the root apex, observation and experiment show clearly that the difference between the permeability of the apices of stem and root is due to the difference in composition of the intervening walls. The importance of this conclusion in relation to the morphology of stem and root is discussed in the next section.

(4) *Morphological Consequences of the Different Permeabilities of Apical Meristems*

Results recorded in other papers have shown that the apical meristems of root and stem are dependent upon supplies received from the vascular system. Some experiments upon the quantitative growth of roots (Priestley and Evershed(23)), have led us to anticipate that should the supply of solute fall below a certain minimal value the rate of growth of the meristematic tissue will be seriously affected.

On comparing the growing points of stem and root from this standpoint and taking into account their relative permeabilities it will be clear that the area supplied will be very different in the two

cases. In the case of the roots, the sap accumulating under pressure in the endodermal cylinder contains supplies of organic materials from the seed reserves or from the leafy area above ground, and is in contact with the closely massed cap of meristematic tissue through which it can diffuse but slowly if at all. It follows that there will be a tendency for the portion of the meristem abutting directly on the endodermal cylinder to be more adequately supplied than the tissue nearer the surface of the root.

On the other hand, at the stem apex it is evident that the supply of sap containing the organic solutes necessary to growth, is not so restricted. Here the endodermis is absent and the intervening walls of the meristem also permit of freer diffusion around the meristematic protoplast. The whole area of the meristem will therefore receive a more proportionate supply of the available solutes. Nothing is known as to the nature of these solutes, but it is probable that they will be amphoteric, nitrogenous compounds in colloidal solution, containing the necessary amino ($-\text{NH}_2$) and carboxyl ($-\text{COOH}$) linkages to allow of their ready employment in synthesis upon adsorption at the surface of the protoplast. It has been found (Priestley and North⁽²¹⁾), that the endodermis, when in the primary stage and possessing only the Casparian strip, permits the ready diffusion of electro-negative colloidal ions whilst it retains those with an electro-positive charge.

In the roots, so far as our observations go, the sap is normally upon the acid side of true neutrality and the hydrolytic products of the reserve proteins of the seed usually have an isoelectric point so far upon the alkaline side that in such a sap they will behave as bases and exist as electro-positive (basic) groups. These groups, then, will be unable to escape from the sap of the endodermal cylinder and will therefore be carried with the sap down to the apical meristem, where they will be as readily absorbed by the meristematic protoplast as was the electro-positive colloidal colour group of the basic dyes (see p. 220). On the other hand, in the stem, the sap, at any rate in the neighbourhood of the growing point, is apparently more alkaline in reaction, so that the same products of protein hydrolysis will now behave as electro-negative or acid groups (Loeb^(10,11)) and as we have seen the meristematic cells of the stem apex are distinguished by their ready staining with acid dyes. These electro-negative groups will be able to escape through the tardily formed endodermis, or its substitute the starch sheath, and here we may have the explanation of the much greater exo-

genous meristematic activity of the cortical region of the stem, as manifested for instance in periderm formation (Priestley and Woffenden (22)).

In the root, then, the meristem directly in contact with the plerome is best nourished and as a consequence its rate of growth is more active than that of the superficial tissue, the outer layers with difficulty supplying enough cortical tissue to keep the plerome cylinder covered. L ndegardh (14) has already pointed out that the periblem appears to be constructed in layers, parallel to the surface, which have been carried past each other in a longitudinal direction by the greater extension of the plerome cylinder within.

Schuepp (24) has discussed the relative rates of growth of the different layers of the meristematic tissue at the stem growing point. As a result of measurement of the relative numbers of dividing cells he has shown that the superficial layers appear to be growing as rapidly as the deep-seated initials directly above the plerome. He points out that it must follow from this fact, that the outer layers of meristem will be unable to accommodate the products of their activity in one plane only and that in addition to increase in superficial area they must be able to increase in depth. Such an increase will lead either to the folding of the surface layer or to the superposition of one cell upon another, at right angles to the surface, which in its effect upon form amounts to the same thing. These folds or extensions, vertical to the direction of normal surface growth, would be the new leaf and stem initials which clothe the surface of the stem apex.

The results recorded by Schuepp seem to us to be the natural consequence of the phenomena previously discussed and the exogenous growth activity of the stem apex to follow from the readiness with which the nutrient solution is able to circulate freely through the tissue and supply equally both outer and inner layers of the meristem. So the rate of growth of both layers remains identical and from the volume relations thus established the lateral outgrowths arise immediately behind the stem apex. The view is therefore put forward, though still requiring much experimental review, that the endogenous and exogenous habit of root and stem may be attributed to the conditions of food supply to the apical meristem.

III. THE ROOT APEX AS AN ABSORPTIVE ORGAN?

In three recent papers Coupin(3,4,5) has brought forward experimental evidence in proof of his theory that the root apex is the chief if not the only organ for the absorption of water and solutes in the root. This view is in such direct opposition to the conclusions we have reached, both on theoretical and experimental grounds, that Coupin's work will now be critically examined, other reasons suggested for his results and some experiments described which, in disproving his suggestions, lend further support to our evidence for the impermeability of the root meristem.

In experiments with seedling peas, carried out in the dark, Coupin(3) shows that whilst such a pea, suspended over water in a small conical flask, fails to grow and dies in a few days, another pea in the same flask with its root apex just immersed in the water grows as normally as could be expected. In a second paper(4) he compares the root development in various seedlings when grown (a) with the tip only in water, and (b) with the whole root submerged. The plants were kept in a humid atmosphere in shade and at a constant temperature of 23° C. In the majority of seedlings experimented upon, growth proceeded much more vigorously when the root tips only were submerged, but in the case of white lupin and *Mirabilis jalapa* L., the roots showed approximately equal growth. Coupin also succeeded in growing the roots of maize and peas with the tips sealed into a narrow tube in which they were kept in moist air, while the rest of the root was completely immersed in water. Under these conditions very little growth took place until the appearance of secondary roots, when Coupin assumed that the root was enabled once again to absorb water through the apical meristem. From these experiments Coupin concludes that roots absorb water exclusively by the apex, the root hairs are assumed to be a protection against too rapid evaporation and to provide the necessary support for the root apex when penetrating the soil.

The following criticisms seem relevant. In the first case, upon the submergence of the first two or three millimetres of the tip in water it seems certain that water will be drawn up the surface of the root and through the cellulose walls of the cortical region by surface tension, so that the normal absorptive region of the root obtains a plentiful supply.

The later experiments are much more striking but it is suggested that they indicate rather the retarding effect of complete immersion

in water upon the growth of a root system, than the impossibility of absorption by this region. There is considerable evidence, often unwillingly obtained in water cultures, that with many plants, the growth of the root system thus immersed is less satisfactory than usual whatever may be the cause. This is particularly true of some of the plants with which Coupin records positive results, notably peas and beans. In the case of plants growing with their root apices inserted by a watertight join into a glass tube containing moist air it is very difficult to be sure that the experimental conditions have not been obtained at the cost of such damage to the root apex as will prevent further growth.

It is possible to demonstrate experimentally that roots will grow when completely out of water. Germinating seeds of *Vicia Faba* L. were taken immediately after the roots had burst through the testa and grown in bottles in the dry air of the laboratory, the necessary water being supplied only through the cotyledons. The roots continued to grow and, although sturdier than usual, attained a length of two or three inches before the outgrowth of the plumules rendered it difficult to continue to supply water to the cotyledons without leakage. It was noticeable that when compared with roots grown in damp sawdust, these roots in dry air produced lateral rootlets more slowly. In the earlier experiments the upper portions of the cotyledons were cemented by plaster of Paris into a wide glass tube, but the continual swelling of the bean broke the plaster of Paris, so that the water leaked on to the root. In later experiments the beans were fixed in position by plasticine made watertight by a layer of 20 per cent. gelatin; the bean growing as shown in text-fig. 2. These roots were very striking structures, clothed to within a short distance of the tip with a coat of short hairs, sturdier, more numerous and persistent than the usual root hairs. These experiments effectually dispose of Coupin's contention that the root will not grow if the apex is out of water, the roots having grown with their whole length in dry air since they had attained a length of a few millimetres.

From a comparison of growth of seedlings with their root tips in distilled water or in Knop's solution, Coupin(5) has attempted to show that salts in solution also enter the plant by the root apex.

But if the water film is drawn up by surface tension to the cortical absorptive region of the root, the salts in solution will be drawn up with it and may enter the root without necessarily passing

through the meristematic tissue. Furthermore, the experiments by Miss Helen Heaps in this laboratory already referred to (p. 213) supply decisive evidence in the case of iron salts, that the salt never enters the living root through the apical meristem but can be traced

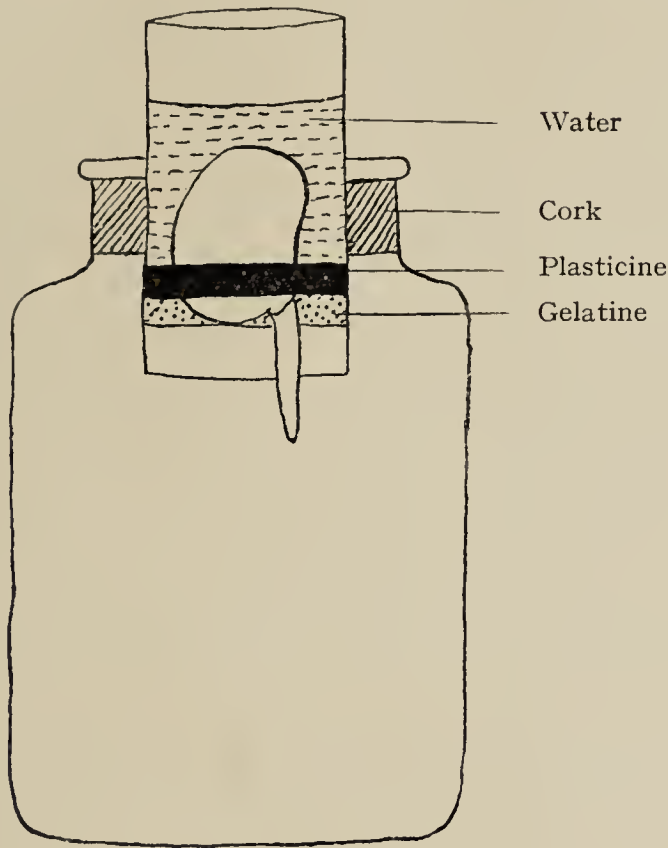


Fig. 2

by appropriate methods, diffusing into the cellulose walls in the absorptive region of the root until held up at the Casparian strip of the endodermis, then diffusing across the endodermal barrier through the protoplast.

SUMMARY

1. Recent work upon the sap pressures existing within the vascular strand leads to the conclusion that the meristematic cap, closing the endodermal cylinder in the young root, must be relatively impermeable to water.

2. It is shown experimentally that this meristematic cap will prevent the passage of water under a pressure of two atmospheres.

3. This meristematic tissue is relatively impermeable to the diffusion of salts in solution and acid dyes but stains very readily with basic dyes.

4. The impermeability of the living meristem is also confirmed by its high resistance to the passage of an electric current.

5. The function of the protoplasts in protoplasmic synthesis is evidence for their impermeability to water.

6. The stem apex allows relatively free diffusion of water and solutes within the cuticle, the meristematic protoplasts at the apex staining readily with acid dyes.

7. The difference in permeability of these meristems is due to the composition of the walls. In the stem apex they are of cellulose, in the root apex they are of unknown substances which are probably in part converted into cellulose by boiling with potash.

8. The sap contained in the endodermal cylinder of the root is apparently more acid than the sap irrigating the stem apex. This fact may be correlated with the retention of colloidal electro-positive ions within the primary endodermis in explaining how in the root the apex obtains the amphoteric substances necessary to growth whilst the cortex receives very little. In the stem those same substances may bear a different charge, and escape more freely into the cortex as well as entering the apical meristem. The different staining capacities of the two apical meristems thus appear very significant.

9. The relative ease with which the apical meristem of the stem is irrigated with substances, as compared with the supply of these substances to the root apex, is shown to suggest a physiological basis for the important morphological differences characteristic of these two growing points.

10. The experimental results, summarised above, are in direct conflict with Coupin's recent work on the function of the root apex. A critical examination of his experiments shows them to be inadequate support for his theory that the growing point is the chief organ of the root for the absorption of water and solute.

BOTANICAL DEPARTMENT,
UNIVERSITY OF LEEDS.

REFERENCES

- (1) BAYLISS, W. M. *Principles of General Physiology*. London, 1915.
- (2) COLE. *Practical Physiological Chemistry*.
- (3) COUPIN, HENRI. Sur le pouvoir absorbant du sommet des racines. *Comptes Rendus*, **168**, p. 519. 1919.
- (4) — Sur le lieu d'absorption de l'eau par la racine. *Comptes Rendus*, **168**, p. 1005. 1919.
- (5) — Sur l'absorption des sels minéraux par le sommet de racine. *Comptes Rendus*, **169**, pp. 242-244. 1919.

- (6) CZAPEK, F. *Biochemie der Pflanzen*. Vol. I. Jena, 1913.
- (7) GARDINER, W. On the constitution of the Cell Wall and Middle Lamella. *Proc. Camb. Phil. Soc.* 5, p. 87. 1884.
- (8) GILSON, EUGÈNE. Recherches chimiques sur la membrane cellulaire des champignons. *La Cellule*, 11, p. 1. 1894.
- (9) JONES, HENRY W. Inorganic Iron in Tissues. *Bioch. Journal*, 14, p. 654. 1920.
- (10) LOEB, JACQUES. Amphoteric Colloids I. Chemical Influence of the Hydrogen Ion Concentration. *Journ. Gen. Physiology*, 1, pp. 39-60. 1918.
- (11) ——— Amphoteric Colloids II. Volumetric Analysis of Ion protein compounds; the significance of the iso-electric point for the purification of amphoteric colloids. *Journ. Gen. Physiology*, 1, pp. 237-254. 1918.
- (12) ——— Amphoteric Colloids III. Chemical Basis of the influence of acid upon the physical properties of Gelatin. *Journ. Gen. Physiology*, 1, pp. 363-386. 1919.
- (13) ——— The Proteins and Colloid Chemistry. *Science*, N.S. 52, pp. 449-456. 1920.
- (14) LUNDEGARDH. Das Wachstum des Vegetationspunktes. *Ber. der deutsch. Bot. Ges.* 32, pp. 77-83. 1914.
- (15) MACALLUM, A. B. On the demonstration of the presence of iron in chromatin by micro-chemical method. *Proc. Roy. Soc.* 50, p. 280. 1892.
- (16) ——— On the distribution of potassium in Animal and Vegetable Cells. *Journ. of Physiology*, 32, p. 95. 1905.
- (17) MANN, GUSTAV. *Physiological Histology*. Oxford, 1902.
- (18) MOLISCH, HANS. *Mikrochemie der Pflanzen*. Jena, 1913.
- (19) MYLIUS, GEORG. Das Polyderm. *Bibl. Botanica*. 18, Heft 79. 1913.
- (20) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytologist*, 19, pp. 189-200. 1920.
- (21) PRIESTLEY, J. H. and NORTH, EDITH E. The Structure of the Endodermis in Relation to its Function. *New Phytologist*, 21, 113-139. 1922.
- (22) PRIESTLEY, J. H. and WOFFENDEN, LETTICE M. Causal Factors in Cork Formation. Paper V in the Series.
- (23) PRIESTLEY, J. H. and EVERSLED, A. F. C. H. A Quantitative Study of the Growth of Roots. *Annals of Botany*, 36, pp. 225-237. 1922.
- (24) SCHUEPP, OTTO. Wachstum und Formwechsel des Sprossvegetationspunktes der Angiospermen. *Ber. der deutsch. Bot. Ges.* 32, pp. 328-339. 1914.
- (25) SHIVE, JOHN W. A Study of Physiological Balance in Nutrient Media. *Physiological Researches*, 1, pp. 327-397. 1915.
- (26) SOLLA. *Æsterr. Bot. Zeitsch.* p. 351. 1879. (Quoted by Gardiner (7).)
- (27) SVEN ODEN. Zur Frage der Azidität der Zellmembranen. *Ber. der deutsch. Bot. Ges.* 34, pp. 648-660. 1916.
- (28) VRIES, DE. Studien over Zingwortels. *Maandbl. vor Natuur-wetensch.* Bd. XIII, pp. 53-86. 1886. Abstract in *Bot. Zeitung*, p. 788. 1886.
- (29) WIELER, A. Die Acidität der Zellmembranen. *Ber. der deutsch. Bot. Ges.* 30, pp. 394-406. 1912.
- (30) WISSELINGH, VAN. Mikrochemische Untersuchungen über die Zellwände der Fungi. *Jahrb. wiss. Botan.* 31, pp. 619-687. 1898.
- (31) ZEMPLÉN, GEZA. *Abderhaldens Biochemische Lexikon*. Vol. 2. P. 527.

LABORATORY NOTES

A SIMPLE RESPIROSCOPE

By SYDNEY MANGHAM

THIS apparatus can be put together easily from common laboratory glassware, and has some advantages over certain other forms in use, *e.g.* that figured in Darwin and Acton's *Practical Plant Physiology*.

A glass drying tower forms the receptacle for the experimental material, *e.g.* germinating peas, which may be enclosed in a loose muslin bag suspended by a thread or supported by the constriction, or the peas may be allowed to rest on a perforated partition placed above the constriction. The top of the tower is fitted with a rubber stopper having two holes, through one of which is passed a thermometer, and through the other a short piece of glass tubing attached to a few inches of rubber tubing provided with a spring clip. In the base of the tower is placed some strong KOH (or water as a control), and the side tubulure is fitted with a rubber stopper connected by glass and rubber tubing to the narrow end of a gently sloping burette of the pinch-cock type. The lower end of the burette rests on the edge of a vessel of water and is provided with a length of rubber tubing which dips down into the water. All the rubber connections must be airtight and should be coated with wax.

To start an experiment release the clip and suck up water to a convenient point in the burette, the graduated side of which should be towards the observer. Although the meniscus is very curved it will be found that a sufficiently good reading can be obtained without difficulty. A sheet of some opaque material should be wrapped round the tower if green tissues are used.

The apparatus gives rapid results owing to (1) the suspension of the material above the KOH, (2) the large surface of KOH available for CO₂ absorption, (3) the small difference in level between the two ends of the burette and the correspondingly small back pull exerted by the water column.

A CHEAP BENCH LIGHT

By SYDNEY MANGHAM

THIS arrangement was devised in order to provide a number of cheap and well protected bench lights for the use of classes doing general microscopic work. In practice it has proved both neat and effective.

A regulation rectangular wooden block carrying a switch and a lamp holder is attached at the back of the bench. A "sentry box" of stout sheet tin is screwed to the sides and to one end of the block round the lamp holder, and is provided with a shade to screen off the light from the eyes of the worker. The back and sides of the "sentry box" are made from one piece of tin and the top and shade from another, the two being soldered together. Greater rigidity is secured by bending over the front edges of the upright sides and of the shade, and also by having a flange of about a quarter of an inch at the sides of the shade. A coat of white enamel inside and of any desired colour outside finishes off the apparatus.

The actual dimensions will vary, but approximately the "sentry box" will be eight inches high and three and a half wide, the shade being adjusted to the width of the bench. Enough space must be left between the top of the box and that of the bulb to allow of lifting the latter for removal.

In the absence of substage rings for blue or ground glass, etc., a screen holder with suitable screen and ventilation holes can be placed in the front of the box by slipping it in between the block and the lower edges of the shade. Studs can be inserted at the base to keep the screen holder secure. The sentry box can also be used conveniently if provided with flexible cord and plug, but for class use it will not be found in the way if fixed permanently.

BOTANICAL DEPARTMENT,
UNIVERSITY COLLEGE OF SOUTHAMPTON.

THE LISTER INSTITUTE NATIONAL COLLECTION OF TYPE CULTURES

WE were asked last year, but have not hitherto found space, to announce that the Medical Research Council has made arrangements to maintain at the Lister Institute, Chelsea Gardens, London, S.W. 1, a National Collection of Type Cultures containing authentic strains of bacteria and protozoa for use in scientific work; and to add to this cultures of fungi of importance in phytopathology, medicine, veterinary science, technology and soil biology, types useful for teaching purposes, and any rare or interesting species. At present only room can be found for those forms with some published distinguishing name or symbol. The British Mycological Society has appointed a fully representative standing committee to consider the ways in which the collection can be made most valuable, and to advise and assist in all questions pertaining to fungi.

The co-operation of bacteriologists and mycologists is earnestly invited, and in return every effort will be made to supply the needs of applicants for cultures. Fully identified species of fungi alone will at present be accepted for the collection, and it should be stated by whom they were named and whether any special medium is required for their growth.

As far as possible cultures will be supplied on demand to workers at home or abroad, a small charge being made to defray the cost of media and postage.

Annual lists of the fungi in the collection will be published in the *Transactions* of the British Mycological Society, and a list of type slides will be kept at the British Museum (Natural History).

All communications are to be addressed to the Curator of the Collection at the above address.

CONTINUATION OF SACCARDO'S *SYLLOGE FUNGORUM*

WE are asked by Dr Montemartini, the Director of the Cryptogamic Laboratory at Pavia, Italy, to announce that two new volumes of this work, carrying it up to 1917, are ready and will shortly be published; and earnestly to request all mycologists to send to him at the Laboratory all published memoirs and especially all descriptions of new genera of fungi, in order that this well known standard work, indispensable to systematic students of the fungi, may be satisfactorily continued.

THE NEW PHYTOLOGIST

VOL. XXI, No. 5

DECEMBER 18, 1922

PERMEABILITY

By WALTER STILES

CHAPTER X

THE INFLUENCE OF EXTERNAL CONDITIONS ON THE INTAKE AND EXCRETION OF WATER BY PLANT CELLS AND TISSUES

THE discussion of the water relations of the plant cell in Chapter IX involved a consideration of the internal conditions which may influence the rate of passage of water through the cell wall and protoplasm. These conditions are the concentration and nature of the substance dissolved in the cell sap which between them determine the osmotic pressure of the cell. The permeability of the protoplasm to solutes will also influence the rate of intake of water, inasmuch as the passage of solutes into or out from the cell will tend respectively to raise or to lower the osmotic concentration of the cell sap and hence the rate of intake of water through the protoplasm and cell wall. Lepeschkin (1908*a*) appears to suggest that the osmotic pressure of a solution is a function of the permeability of the membrane, but the error of this view is obvious and has already been indicated by Brooks (1917*a*), Höfler (1920) and V. H. Blackman (1921). Apart from electrical effects, the osmotic pressure which can be developed with a permeable membrane must continually fall with time until equilibrium of the penetrating solute on the two sides of the membrane is attained.

External conditions which are known to influence the rate of passage of water into and out from the cell are temperature and the composition of the external medium. It has to be noted that changes in the rate of passage of water into and out from the cell are not necessarily to be attributed to changes in the permeability of the protoplasm. For, neglecting the possible effect of limiting plasma-membranes, the rate of intake or excretion of water is the resultant

of (1) effects attributable to purely osmotic relations of the cell, (2) effects due to the entrance or exosmosis of dissolved substances which alter the osmotic relations, (3) effects on the capacity for imbibitional swelling of cell wall, protoplasm and vacuole, and (4) effects resulting from any influence on the permeability of the cell wall and protoplasm. It may not always be possible to distinguish with certainty which of these various effects is or are mainly responsible for observed behaviour. It will therefore be most satisfactory first to describe the results obtained experimentally, and then to discuss how far these observed results are to be correlated with protoplasmic permeability or with other phenomena indicated above.

Temperature

A number of earlier observations have suggested that the rate of intake of water by, or its excretion from, plant cells and tissues, is considerably influenced by temperature. Among these may be mentioned those of Vesque (1877) on the absorption of water by forest trees, Wieler (1893) on the bleeding of cut stems of a number of plants, and Lepeschkin (1906) on the exudation of water by the sporangiophores of *Pilobolus*. As these results, suggestive as they are, do not provide us with exact data for our analysis, we may pass at once to a consideration of those researches in which exact measurements of the effect of temperature on water intake or excretion have been attempted.

DETERMINATIONS BY THE PLASMOLYTIC METHOD

Systematic attempts to determine the influence of temperature on the rate of passage of water through the cell membranes were made by Krabbe (1896) and van Rysselberghe (1901). The former measured the time required for complete contraction of cylinders of turgid pith when plasmolysed at different temperatures and concluded that the rate of excretion and absorption of water rises with increase of temperature.

Van Rysselberghe used cylinders of elder pith, epidermal cells of *Tradescantia* and *Begonia*, *Lemna*, *Spirogyra* and other plants. The cylinders of pith were plasmolysed at different temperatures in strong sucrose solutions (0.731 *M*) and the amount of shrinkage measured at different times over a period of 24 hours. The temperature effect was determined by comparing the amount of shrinkage after two hours at the different temperatures. But obviously this

could only give a correct indication of the temperature effect if the relative amounts of shrinkage at the different temperatures were the same at all times during the experiment, which was not the case. Had he chosen to compare the shrinkage after three or four hours instead of after two, he would have obtained a quite different set of values for the relation between temperature and rate of passage of water through the cell membranes. The only way to obtain correct information on the influence of temperature in these experiments is to compare the *rate* of passage of water out of the tissues at different temperatures, but under otherwise exactly the same conditions. This is to be accomplished, as pointed out by Miss Delf (1916), by constructing the time-shrinkage curves for the different temperatures and then comparing the rate of shrinkage *at the same stage of shrinkage*, as given by the value of $\tan \alpha$, where α is the angle made by the tangent to the curve with the time axis.

The data of van Rysselberghe have been subjected to this treatment by Miss Delf, when they indicate an increase in the rate of passage of water through the cell membranes with increasing temperature up to about 20° C., but between this and 30° C. the effect of temperature is negligible. Miss Delf found that such a result is obtainable only when a very strong plasmolysing solution is used, the reason being apparently that there is an "upper limit to the rate at which the mechanical tissue system of cells can collapse and shrink," so that with the strong solutions used by van Rysselberghe a great part of the contraction curves obtainable from his data represent a contraction of the cell wall system over which permeability has no control.

Other experiments of van Rysselberghe, in which only the times taken for cells to plasmolyse or deplasmolyse are given, are obviously open to still greater objection, both on the grounds already stated in the case of the experiments with pith, and also on account of the difficulty of carrying out such experiments with exactitude. Further, it is doubtful whether sufficient account has been taken of the great variability of plant tissue from different individuals of the same species, so that it is not certain whether van Rysselberghe's results could be accepted as expressing more than approximately the relation between temperature and rate of intake or excretion of water, even if they were not open to grave objection on other grounds. No further consideration will therefore be given to the results of Krabbe and van Rysselberghe in this place.

DETERMINATIONS OF THE EFFECT OF TEMPERATURE BY THE METHOD
OF THE RATE OF LINEAR SHRINKAGE OF TURGID TISSUES

The first critical research on the influence of temperature on cell permeability to water is that of Miss Delf (1916). Her method was similar in principle to that used by van Rysselberghe, but was considerably more sensitive. By means of the optical lever, which had already been introduced into plant physiology by Bose (1906) to record small movements in plants, changes in length of short strips of tissue could be observed under a magnification of 350 diameters. The apparatus devised by F. F. Blackman and used by Miss Delf is directly applicable only to the investigation of hollow cylindrical structures, those used by Miss Delf being onion leaves and dandelion scapes.

The essentials of the method are as follows. The lower end of the plant cylinder is firmly fixed by means of cotton to a glass tube of narrow bore which passes through a cork held immovable by a metal clamp. The upper end of the cylinder is pierced by a fine glass hook attached to a loop of very fine waxed cotton which at its upper end is itself attached to the projecting wire arm of the optical lever, which carries a small mirror reflecting the image of an illuminated cross wire on to a millimetre scale. Contraction of the cylinder can thus be very accurately measured by the movement of the image of the cross wire on the scale. For details of the apparatus and procedure, including the method adopted for obtaining different constant temperatures, reference must be made to the original paper.

The material selected for investigation already noted above was chosen because the inner surface of the hollow cylinders of which it is composed is lined entirely with cells possessing thin cellulose walls. Solutions of cane sugar in various concentrations (0.18, 0.3, 0.5 and 0.731 weight-molecular solutions) were allowed to flow through the hollow cylinders of plant tissue and the contraction resulting followed by the optical lever arrangement. The total contraction produced by these solutions varies somewhat with the age of the material, but very greatly with the concentration of the solution. Thus dandelion scapes in 0.30 *M* sucrose contracted to the extent of 0.2 to 0.4 per cent. of the original length, while in 0.731 *M* sucrose the contraction was as much as 2 to 2.5 per cent. of the original length. Similarly, onion leaves in 0.18 *M* sucrose contracted 0.3 to 0.5 per cent. and in 0.731 *M* sucrose 3 per cent.

of the initial length. Although greater contractions were obtained in the stronger sucrose solutions, it was found almost impossible to decide how much of this was to be attributed to plasmolysis, and how much to the after-shrinkage of the cell walls. On this account weaker solutions, "subtonic," that is, diluter than isotonic, were used for the crucial experiments, the actual concentrations chosen being such that the contractions produced after the tissue had been allowed to swell to equilibrium in pure water were sufficiently large to be measured satisfactorily with the apparatus employed, while a definite end-point was reached in about two hours. The solutions which fulfilled these conditions were 0.18 *M* sucrose for onion leaves and 0.3 *M* for dandelion scapes.

With the subtonic solutions employed the shrinkage was found to follow an approximately logarithmic course. Miss Delf states that "with great care in the choice of material and in repeating the same experimental conditions, there was a certain amount of variation in the rate of plasmolysis at the same temperature. At medium temperatures, therefore, it was usual to perform several experiments under the same conditions, and to select the ones which gave the curve of approximately logarithmic form." This selection of certain curves appears a weakness in Miss Delf's work, for as the rejected experiments were conducted with the same care and under the same conditions as the selected experiments, the form of the time-shrinkage curves does not seem an adequate ground for selecting some and rejecting others.

At temperatures above that of the laboratory complications occur. These are (1) a contraction resulting from the effect of prolonged high temperature, probably on account of the escape of water from the cells as a result of increased exosmosis of solutes from the cell sap at high temperatures, and (2) an expansion resulting probably from the entry of sucrose into the cell. Up to 35° C. in the case of the dandelion scape, and up to 36° C. in the onion leaf, it was found to be unnecessary to apply any correction on account of these complications, but above this temperature the shrinkage due simply to the presence of the subtonic solution at the higher temperature was obtained by subtracting the shrinkage resulting in distilled water at the higher temperature from the total shrinkage actually observed.

From the shrinkage-time curves obtained by Miss Delf the rates of contraction at 30, 50 and 70 per cent. of the total contraction were calculated for the temperatures employed in the experiments, and the rates for temperatures between 5° and 40° C. found by inter-

pulation. The relative values for the rate of linear shrinkage of the tissue at the stage of half-contraction are shown in Table XXVII, the values for 25° C. being taken as unity.

TABLE XXVII

Relative rates of linear contraction of onion leaves and dandelion scapes in subtonic solutions of sucrose at different temperatures.
(Data from Miss Delf)

Temperature in centigrade degrees	Relative rate of linear contraction	
	Onion leaves in 0.18 <i>M</i> sucrose	Dandelion scapes in 0.3 <i>M</i> sucrose
5	0.36	—
10	0.44	0.22
15	0.50	0.30
20	0.66	0.50
25	1.0	1.0
30	1.7	1.9
35	2.9	3.0
40	5.0	5.0

From these numbers the temperature coefficients (Q_{10} , see Chapter III, p. 101) for a rise of 10° C. were calculated, with the results given in Table XXVIII.

TABLE XXVIII

Temperature coefficients for rate of linear shrinkage of onion leaves and dandelion scapes in subtonic sucrose solutions.
(Data from Miss Delf)

Range of temperature in centigrade degrees	Temperature coefficient (Q_{10})	
	Onion leaves	Dandelion scapes
5-15	1.4	—
10-20	1.5	2.3
15-25	2.0	3.3
20-30	2.6	3.8
25-35	2.9	3.0
30-40	3.0	2.6

It is particularly interesting to consider how far these temperature effects are to be regarded as effects of temperature on permeability of the protoplasm, as they are assumed to be by Miss Delf.

If the permeability of the cell or tissue to water is defined as the quantity of water passing through unit area of the cell membranes (cell wall + protoplasm) in unit time under unit difference of pressure we have the equation

$$\frac{dw}{dt} = \zeta (P_e - P + T) A,$$

where $\frac{dw}{dt}$ is the rate of passage of water out of the cell or tissue, ζ is the permeability, P_e and P are the osmotic pressures of the external solution and the cell sap respectively, T the turgor pressure (cf. Chapter IX), and A is the area of the cell surface through which water passes.

Now the rate at which water is lost by the tissue is proportional to the rate of volume shrinkage, and if c.g.s. units are employed may be taken as equal to it. Let us suppose that in a small time interval δt the length l of the cylinder contracts by a small length δl , and that in the same time the contraction in width is δa , the original diameter of the cylinder being r and the thickness of the tissue a . Let us further suppose the water loss in the same time is δw . The change in volume in the time δt is then

$$\pi a l (2r - a) - \pi (a - \delta a) (l - \delta l) \{2(r - \delta a) - (a - \delta a)\}$$

or
$$\pi [a(2r - a)\delta l + 2rl\delta a] - (l\delta a + 2r\delta l + \delta a\delta l)\delta a].$$

Hence, using c.g.s. units, we have in the limit the rate of water loss by the tissue given by

$$\frac{dw}{dt} = \pi a (2r - a) \frac{dl}{dt} + 2\pi r l \frac{da}{dt}.$$

Whence
$$\zeta = \frac{\pi}{P_e - P + T} \left\{ a(2r - a) \frac{dl}{dt} + r l \frac{da}{dt} \right\} \frac{1}{A}.$$

Thus, if it is assumed (1) that differences in imbibitional swelling at different temperatures are negligible, (2) that the value of the exudation pressure $P_e - P + T$ at the same stage of shrinkage is practically constant for temperatures over the range 5° to 42° C., and (3) that the cylinders shrink only in a longitudinal direction, the above equation may be written

$$\zeta = K \frac{dl}{dt},$$

where K is a constant since A is the same for all cylinders in the same stage of shrinkage. If, on the other hand, there should be a shrinkage in a transverse direction proportional to the longitudinal shrinkage, the equation connecting permeability and shrinkage may be written

$$\zeta = K l^2 \cdot \frac{dl}{dt},$$

K again being a constant.

Now the first two assumptions are very reasonable, and it is probable that the actual shrinkage would approximate to one of the two cases considered under (3). In the first of these cases the

permeability is proportional to the rate of linear shrinkage, in the second to the rate of linear shrinkage multiplied by the square of the length. Hence if comparisons are only made, as in the case of Miss Delf's experiments, with cylinders in the same stage of shrinkage, l^2 will be the same in all cases compared with one another and the permeability will similarly be proportional to the rate of linear shrinkage.

The values obtained by Miss Delf may then legitimately be regarded as measures of the permeability of the cell membranes (cell wall + protoplasm) to water. Miss Delf speaks of them as measures of the permeability of the protoplasm, but as the effects of cell wall and protoplasm are not separated, they are better regarded as measures of the permeability of the cell. It will be noted that errors arising from exosmosis of dissolved substances and intake of salt are allowed for in the experimental procedure, and need not therefore be further considered.

DETERMINATIONS OF THE INFLUENCE OF TEMPERATURE ON THE RATE OF INTAKE OF WATER BY THE CHANGE IN WEIGHT METHOD

Storage Tissues. The influence of temperature on the intake of water by storage tissues, potato tuber and carrot root, has been investigated by Stiles and Jørgensen (1917 *b*). In the method employed by them, the course of absorption of water at different temperatures is followed by immersing the tissue of known weight in water and weighing the tissue at intervals. The change in weight may be fairly attributed to gain or loss of water only, as the weight of dissolved substance which diffuses out from the tissue during the period of the experiment is negligible in comparison with the weight of water involved.

In order to eliminate errors due to the variability of tissue in different samples, a variability which may be very large, and to ensure equality of surface and weight of tissue in all comparative experiments, the following procedure was adopted. Cylinders of tissue were cut from the tubers or roots by means of a cork-borer 1.8 cms. in diameter. These cylinders were then cut into disks of a uniform thickness of 0.2 cm. by means of a hand microtome. For any series of experiments performed for comparative results all the disks required were cut at once, well mixed together and weighed in sets of 20. In this way samples of the material, each sample consisting of 20 disks, were obtained having approximately

the same weight and surface. The experiments at any one temperature were all conducted in triplicate or quadruplicate, so that for a comparison of the rate of water intake at four different temperatures, 12 or 16 sets of 20 disks were required, 3 or 4 at each temperature. The probable error of the determinations was calculated so that some idea of the degree of reliability of the results might be obtained. The temperatures were maintained constant by means of thermostat baths provided with gas-mercury-toluene regulators.

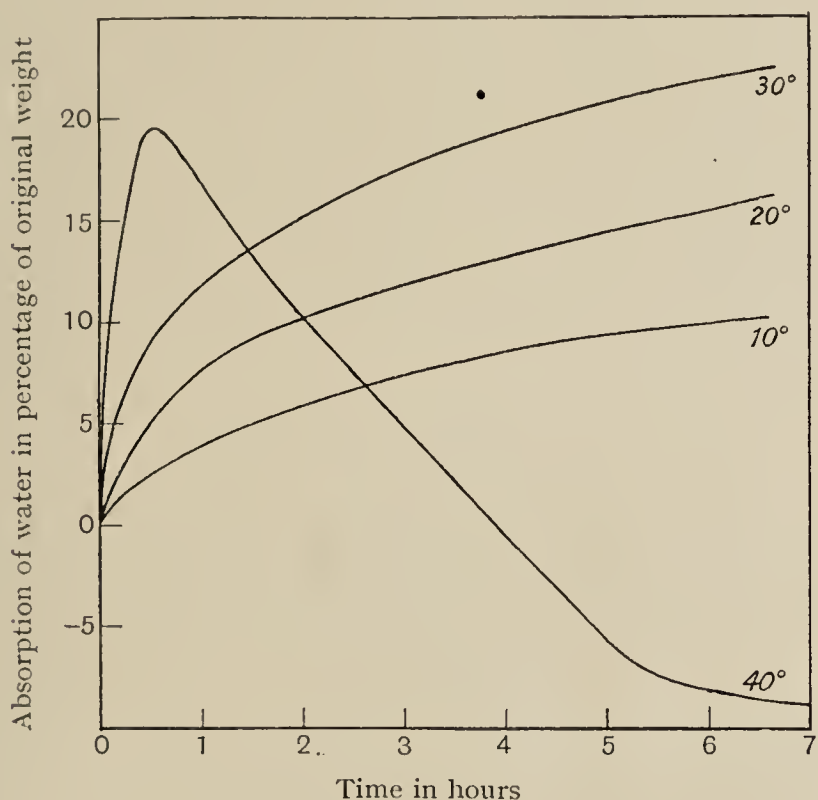


Fig. 7. Curves illustrating the influence of temperature on the absorption of water by potato tuber. (After Stiles and Jørgensen.)

The course of water absorption at different temperatures by potato and carrot is shown in Figs. 7 and 8. From these absorption-time curves the rate of swelling of the tissue has been determined in the same way as the rate of shrinkage in Miss Delf's experiments, that is, by measuring the tangent of the angle made with the time axis by the tangent to the curve at any particular stage of swelling. By comparing the rate of intake of water at different temperatures at the same stage of swelling, the following temperature coefficients for the rate of intake of water are obtained.

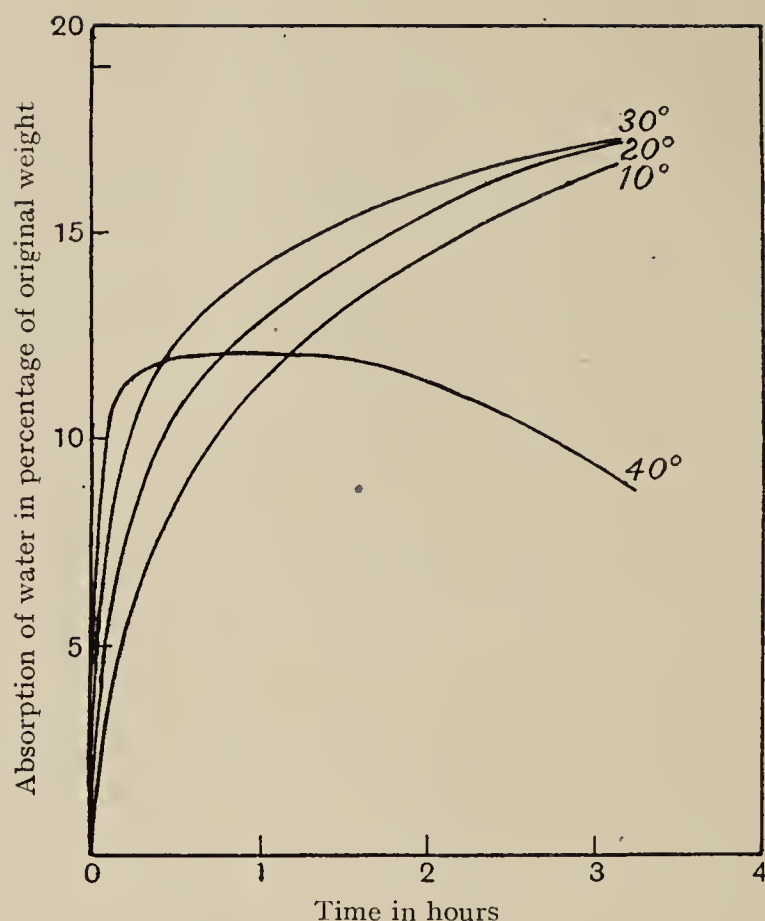


Fig. 8. Curves illustrating the influence of temperature on the absorption of water by carrot root. (After Stiles and Jørgensen.)

TABLE XXIX

Temperature coefficients of the rate of intake of water by storage tissues. (Data from Stiles and Jørgensen)

Temperature range in centigrade degrees	Temperature coefficient (Q_{10})	
	Potato tuber	Carrot root
10-20	3.0	1.3
15-25	2.75	1.4
20-30	2.7	1.6

Two points deserve special notice in regard to the results of Stiles and Jørgensen. In the first place, the rate of swelling of carrot was found to be considerably greater than that of potato. Thus at 10° C. the rate of swelling of carrot was about nine times that of potato under apparently the same conditions with regard to degree of swelling, while at 30° C., in spite of the lower temperature coefficient in the case of carrot, the rate of swelling was still a little

more than twice as great in carrot as in potato. This is probably due in part to the higher suction pressure observed in the carrot tissue used (cf. Chapter IX), but as there is no evidence that the tissues were in the same absolute stage of swelling, too much stress must not be laid on the difference between the two tissues.

In the second place a phenomenon noted by Miss Delf in onion and dandelion appears also in carrot and potato tissue, namely, the contraction and loss in weight of the tissues at temperatures above 30° C., which probably corresponds to a rapid exosmosis of solutes from the cell. This breaking down of the semi-permeability of the protoplasm requires some time to become obvious, so that, as the curves for 40° C. show in both potato and carrot, there is first an initial period of rapid absorption of water followed by exudation. This phenomenon has also been observed in other storage tissues, such as red beetroot and artichoke tubers (*Helianthus tuberosus*).

Confining attention only to the curves where this complication is not present, and using the same notation as in the preceding section, we have the relation

$$\frac{dw}{dt} = \zeta (P - T) A,$$

where it is assumed as before that the effect of temperature on imbibitional swelling is negligible. If it is also assumed as before that $P - T$ is approximately constant over the range of temperature in question, the temperature coefficients given above may be accepted as those of the permeability of the cells of the tissues to water.

Seeds. The influence of temperature on the absorption of water by barley grains was investigated by A. J. Brown and Worley (1912) by means of the change in weight method. Known weights of grains were immersed in water kept at temperatures of 3·8° C., 21·1° C. and 34·6° C., and the increase in weight determined after various intervals of time. From the absorption-time curves so obtained the rate of water intake at different stages of absorption was measured for the three temperatures employed. The values obtained indicated that the rate of water absorption at 21·1° is about 3·40 times the rate of absorption at 3·8°, while the rate at 34·6° is about 2·44 times that at 21·1°. These numbers give a mean value for the temperature coefficient (Q_{10}) of about 2 (actually 1·99), a value characteristic of a number of chemical reactions. Somewhat lower values were obtained by Shull (1920) in a series of carefully conducted

experiments with seeds of *Xanthium pennsylvanicum* and small Scotch yellow split pea, the values for Q_{10} between 5° and 35° C. found for *Xanthium* being 1.55 in one case, and 1.83 in another, and for pea 1.6. Very similar, though on the whole lower values, were obtained by Denny (1917 a) for the temperature coefficient of the rate of passage of water through the detached seed coat of *Arachis hypogaea* (1.34 to 1.64).

Brown and Tinker (1916 a) regard the rate of entry of water into barley grains as given by the equation

$$\frac{dy}{dt} = k(a - y),$$

or, integrating,

$$\log \frac{a}{a - y} = kt,$$

where y is the quantity of water absorbed in a time t , and a and k are constants. Shull, however, holds that both his own experimental results and those of Brown and Worley are better expressed by the equation

$$y = a \log_{10} (bt + 1) + c,$$

where y is the quantity of water absorbed, t the time of immersion and a , b and c are constants. For the whole course of water intake the time-absorption curves can, in some cases at any rate, best be represented by three such equations representing the early, middle and late stage of water absorption. Apart from their value in enabling the temperature coefficients to be calculated with exactitude, these equations do not help us much in explaining the processes of water intake.

In the case of seeds, where the percentage of water is comparatively low and the percentage of colloidal substances susceptible to imbibitional swelling very high, it is clearly impossible to describe the temperature coefficient of water absorption as a temperature coefficient of the cell membranes. To determine the effect of temperature on the different processes involved in the water intake by seeds, further experimental work is necessary. Denny's work with seed coats separated from the seeds suggests a way in which this might be attempted. Sufficient data are, however, at present lacking.

ON THE VALUES OF THE TEMPERATURE COEFFICIENT OF THE
PERMEABILITY OF THE CELL TO WATER

Three points deserve special mention in regard to the temperature coefficient of the cell membranes to water.

1. The permeability to water increases continuously from the lowest to the highest temperatures investigated. This means that, other factors being equal, water absorption or excretion increases continuously with rise of temperature. Above 30° C. a "time factor" (F. F. Blackman, 1905) appears, inasmuch as with prolonged immersion in water at such higher temperatures the initial absorption is followed by excretion of water; this has been observed in onion leaves, dandelion scapes, tubers of potato and artichoke, and roots of carrot and red beet.

2. The temperature coefficients vary considerably for different tissues and over different temperature intervals in the case of the same tissue, the lowest observed being for carrot root and the highest for dandelion scapes.

3. The temperature coefficients are higher, and often considerably higher, than those found for a physical process such as diffusion of solutes in water, which are generally between 1.2 and 1.3, and approach instead more closely those characteristic of chemical processes. Yet there is no ground for supposing that we have to do here with a chemical rather than a physical process. The results rather suggest the necessity for caution in drawing conclusions with regard to the nature of a process from the value of the temperature coefficient.

Brown and Worley point out that an exponential increase in the value of a physical property is very rare, but that vapour pressure of water is an exception. The general equation

$$v = ae^{k\theta}$$

gives both the relation between vapour pressure and temperature and that between water absorption by barley grains and temperature. Shull, however, does not agree that the rate of intake of water by seeds is determined by vapour pressure. He is no doubt correct in supposing that in the case of seeds the rate of water intake is determined by a number of factors, among which chemical changes in the colloids of the seed coat may not be unimportant. Denny also, on account of the values of the temperature coefficient found by him, disagrees with Brown and Worley in regard to vapour pressure of water determining the rate of entrance of water into seeds.

Dissolved Substances

The investigation of the influence of dissolved substances on the intake and exosmosis of water by plant tissue (potato tuber and carrot root) has also been investigated by Stiles and Jørgensen (1917 *b*) using the change in weight method. Some further observations have been made by Thoday (1918 *a*) using the same method in a cruder form. As the results obtained show that the influence of all dissolved substances is not the same, it will be convenient to consider the different cases separately.

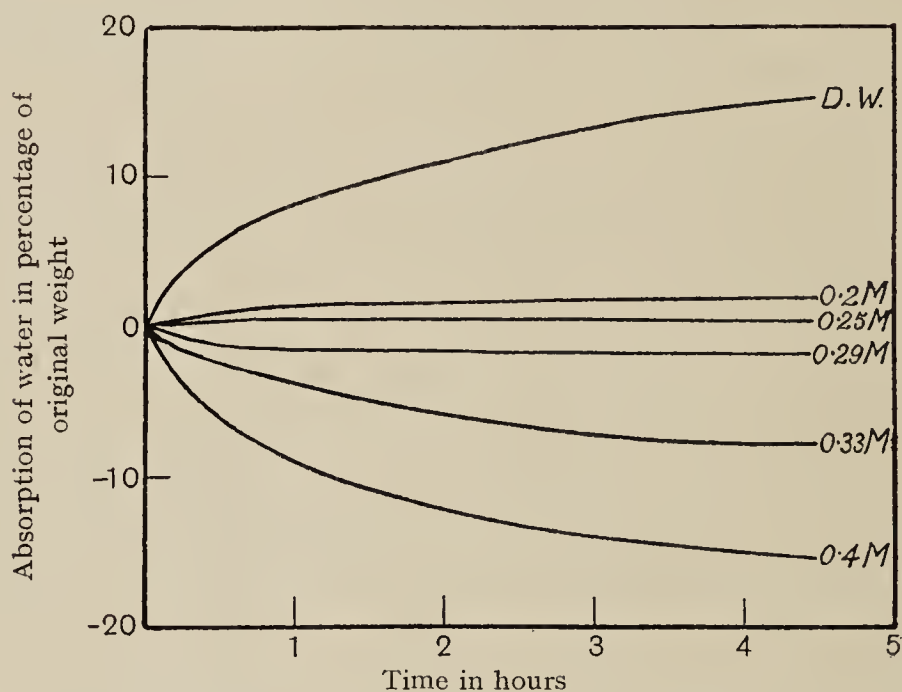


Fig. 9. Curves illustrating the absorption of water by potato tuber immersed in distilled water and sucrose of various concentrations (0.2 *M* to 0.4 *M*). (After Stiles and Jørgensen.)

I. SUCROSE

Experiments were conducted on potato tissue at 20° C. with the same technique as that already described. Sucrose solutions of various concentrations were employed, the absorption-time curves for the tissue in the different concentrations of sucrose being shown in Fig. 9. It will be observed that with increasing concentration of sucrose both the rate of water intake and the total amount of water absorbed are reduced, and that in a solution of about $M/4$ concentration the intake of water is approximately zero. With higher concentrations of the sugar exosmosis of water takes place, both the

rate of exosmosis and the quantity of water exuded increasing with increase of sugar concentration.

Since, as we have already noted, the rate of intake of water is proportional both to the permeability and to the net suction pressure, so that

$$\frac{dw}{dt} = \zeta (P - P_e - T) A,$$

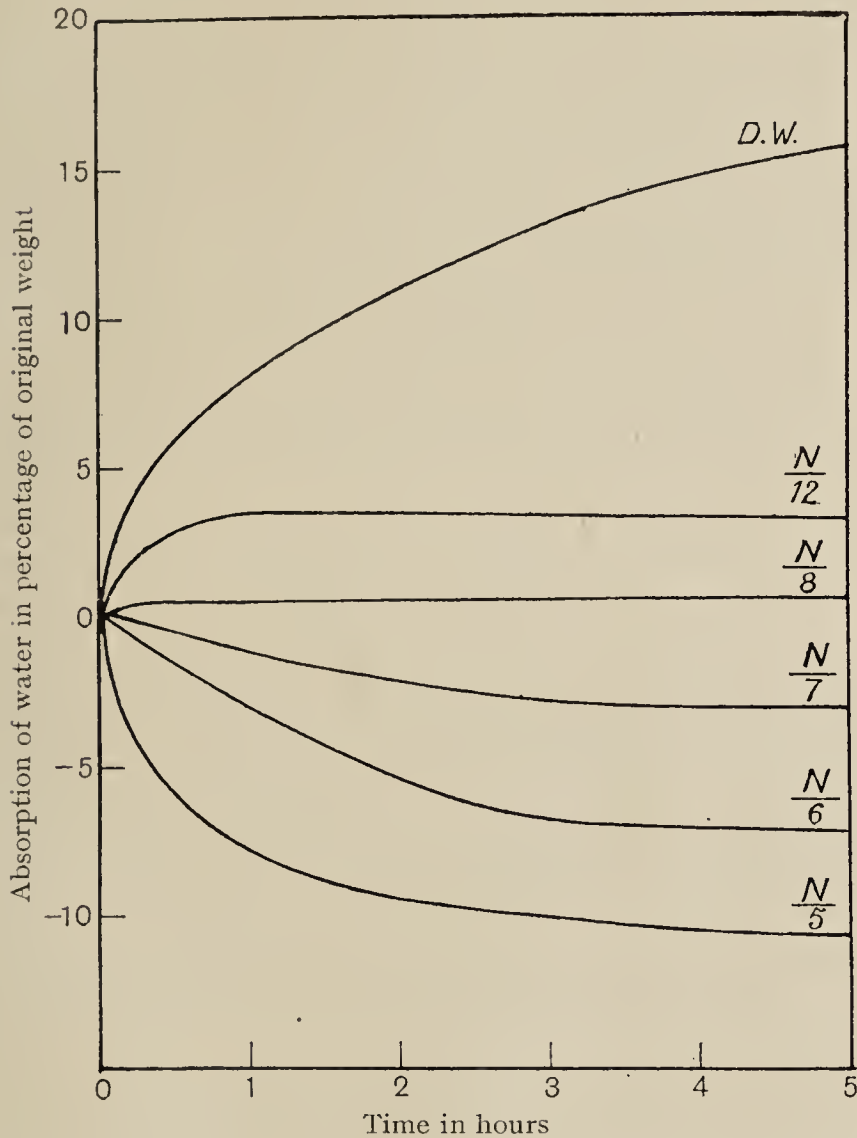


Fig. 10. Curves illustrating the absorption of water by potato tuber immersed in distilled water (*D.W.*) and sodium chloride solutions of various concentrations ($\frac{N}{5}$ to $\frac{N}{12}$). (After Stiles and Jørgensen.)

and as with increase in the concentration of the sugar P_e increases, and consequently $P - P_e - T$ decreases, the observed results can be explained on account of the reduction of the net suction pressure with increasing concentration of the external sugar solution; there

is no evidence from existing data that the presence of the sugar has any influence on the permeability of the cell membranes to water, nor is there any evidence of an appreciable entrance of the sugar nor of exosmosis of solutes from the cell sap. Similar results with potato in sucrose solutions were obtained by Thoday (1918 *a*).

2. SODIUM CHLORIDE

Similar experiments with sodium chloride showed that this salt behaved to potato tissue in the same way as sucrose, but that the solution in which the tissue neither gained nor lost in weight possessed a concentration slightly higher than $N/8$. This lower concentration is to be expected from theoretical considerations as the ionisation of the sodium chloride in solution almost doubles its osmotic pressure. The absorption-time curves for the tissue in different concentrations of the salt are shown in Fig. 10.

Similar experiments with carrot tissue showed that in a solution of $N/3$ concentration this tissue neither gained nor lost in weight.

3. ETHYL ALCOHOL, OCTYL ALCOHOL, CHLOROFORM AND MERCURIC CYANIDE

The results obtained with ethyl alcohol were quite different from those with sucrose and sodium chloride. The results with potato tissue are shown graphically in Fig. 11. With lower concentrations (up to molecular) absorption at first takes place as in dilute solutions of sucrose. But it will be observed that if ethyl alcohol behaved similarly to sucrose, we should expect the potato to undergo neither gain nor loss in weight in a solution having a concentration of about $M/4$, whereas swelling even takes place initially in solutions having as high a concentration as $2M$. But in concentrations M and $2M$ this swelling reaches a maximum, after which the solution loses water. With $M/2$ alcohol the maximum was not reached even after 16 hours, but the swelling was lower than in distilled water; in M alcohol the maximum intake of water is reached in about 7 or 8 hours, while in $2M$ alcohol in about half an hour. With concentrations of alcohol higher than $2M$ any preliminary period of water intake was not detected by the method, the excretion of water being rapid, the more so the higher the concentration of the external solution. The observation of Overton (1895) that ethyl alcohol may enter the cell so rapidly from hypertonic solutions that plasmolysis does not take place is in agreement with the behaviour noted here.

The behaviour of a secondary octyl alcohol was similar, and the curves given by Thoday for chloroform and mercuric cyanide leave little doubt that these substances behave on the whole similarly to the alcohols examined.

These results are simply explained. It is clear that these substances bring about an increase in the permeability of the cell to substances dissolved in the cell sap (Czapek, 1910 *a*; Stiles and Jørgensen, 1917 *a*), while at the same time it is to be supposed that the solute in the external liquid is itself capable of entering the cell.

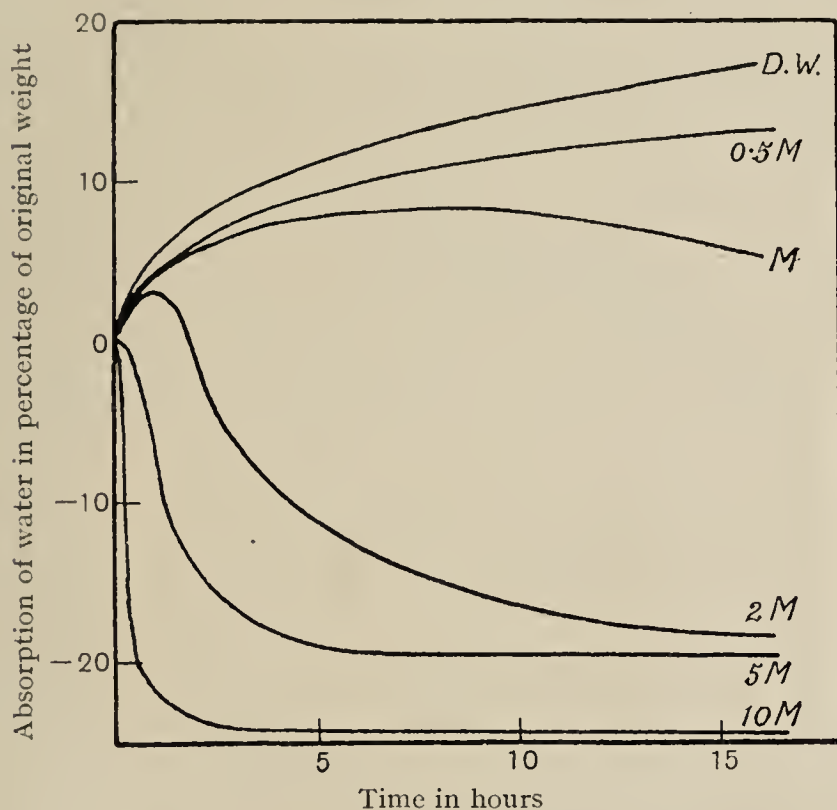


Fig. 11. Curves illustrating the influence of ethyl alcohol in concentrations 10 *M* to 0.5 *M* on the absorption of water by potato tissue. (After Stiles and Jørgensen.)

In the first stage of the experiment there will thus occur a rise in the osmotic pressure of the cell-sap on account of the addition to it of alcohol or other substance. If the permeability of the cell membranes remained unaltered we may suppose that the swelling in the most concentrated of the alcoholic solutions would be the same as that in distilled water at equilibrium, but the rendering permeable of the cell to the solutes in the cell sap brings about a diminution of osmotic pressure within the cell which accordingly loses water on account of this. Thus to the first stage of excessive swelling there follows a

stage of water loss as the membranes are rendered more permeable. The exact shape of the absorption-time curve in any particular case must depend on the concentration of the external solution, the permeability of the cell to the external solute and the rate at which the solute acts on the cell membranes in rendering them permeable to the solutes of the cell sap. Thus it is not to be expected that the behaviour of the tissue in regard to water gain or loss will be the same with different toxic substances, nor is this the case.

4. SULPHURIC ACID, OSMIC ACID AND MERCURIC CHLORIDE

These substances influence the swelling of potato rather similarly to those just discussed, that is, there is a preliminary period of

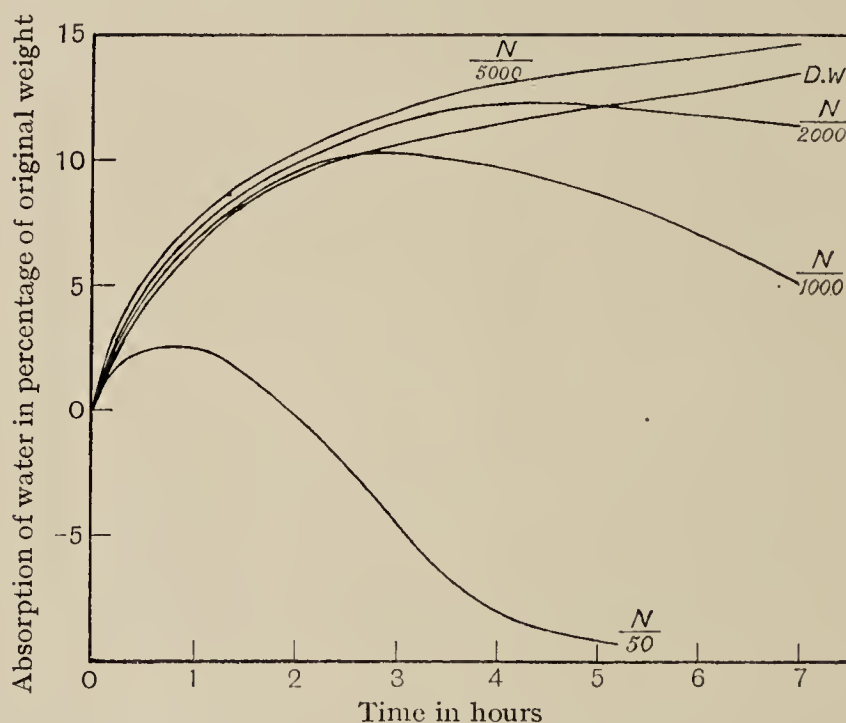


Fig. 12. Curves illustrating the influence of sulphuric acid on the absorption of water by potato tuber. (After Stiles and Jørgensen.)

swelling followed by a period of shrinkage. There is no doubt that acids are absorbed by plant tissue, the evidence for which will be discussed in a subsequent chapter. The results obtained for sulphuric acid are shown in Fig. 12. It will be observed that in a concentration of $N/50$ the intake of water reaches a maximum in about an hour, while in a concentration as low as $N/2000$ the swelling gives place to shrinkage after 4 or 5 hours. Even in $N/5000$ sulphuric acid shrinkage supervened before the lapse of 24 hours while in distilled water the tissue was still undergoing slow swelling after this lapse of time.

A curious phenomenon noted in the case of sulphuric acid is that in the lower concentrations the tissue actually absorbed water at more rapid a rate at first than in distilled water. Calculation of the probable error of experiment left no doubt that this is not to be accounted for on account of the variability of different samples of tissue. This phenomenon is rather difficult to account for, since as the concentration of acid external to the tissue is so low, the penetration of it into the cell sap could scarcely increase the osmotic pressure of the latter sufficiently to account for the increased rate of water absorption. It is possible that this increased swelling may be referred to the increased absorption of water by proteins and other colloidal systems in acid solutions as compared with distilled water, but definite evidence confirmatory of such a view is wanting.

Thoday (1918 *a*) has shown that a similar increase of rate of water absorption in lower concentrations of the solute as compared with that in distilled water is to be observed in the case of osmic acid and mercuric chloride. It may be significant that mercuric chloride undergoes partial hydrolysis in solution so that the solution contains a small quantity of hydrochloric acid.

5. ETHYLIC ACETATE AND PHENOLS

The absorption of ethylic acetate by grains of barley was examined by Brown and Worley (1912). Ethylic acetate enters the grain, but the rate of water intake is more rapid than from pure water. This was regarded by Brown and Worley at the time as possibly due to a simplification of the water molecule in presence of this ester. Later Brown and Tinker (1916 *a*) observed a similar phenomenon in the case of various phenolic solutions. Barley grains absorbed various solutions of phenol of different concentrations the more rapidly the more concentrated the solution. With a number of different phenols in half-normal concentration the rate of absorption was in this order: phenol, catechol, resorcinol, pyrogallol. The last entered at the same rate as water. Since variations in osmotic pressure, vapour pressure and viscosity of these various solutions are slight, while the rate of absorption is in inverse order to the surface tensions of the solutions (Worley, 1914), Brown and Worley conclude that surface tension is also a factor in determining the rate of entrance of solutions into barley grains.

PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

V. CAUSAL FACTORS IN CORK FORMATION

By J. H. PRIESTLEY AND LETTICE M. WOFFENDEN

CONTENTS

	PAGE
Introduction	252
I. The Formation of Wound Cork	253
II. Leaf Fall	261
III. Normal Cork and Lenticel Formation	262
Summary	266
References	267

INTRODUCTION

WE propose to attempt in this paper a reconsideration of the phenomena connected with cork formation under the various conditions in which it is known to occur within the plant. The attempt will involve the brief re-statement of the results of many other investigators, but this repetition seems worth while as it is hoped to show that in every case, the formation of cork follows from certain causally connected phenomena, a sequence of causation which so far as we are aware has not been traced previously.

We have been led to examine cork formation from this point of view as the result of other work which was proceeding in the laboratory, and which began with a study of the mechanism of sap exudation in the phenomenon of root pressure(19). A wider extension of the investigation has shown that sap pressures may arise within the vascular strands as the result of the activity of parenchymatous tissues in root, stem or leaf(21). At the same time other observations(23, 24) have directed attention to the importance of this sap pressure in initiating and sustaining the activity of meristematic tissues. Such tissues are always parenchymatous tissues irrigated by the sap from the vascular strand. This sap supply is in many cases controlled and contained by the limiting cylinder of the endodermis (Priestley and North(22)), and in such cases the meristematic activity of parenchymatous tissues external to the endodermis may be strictly limited.

When we apply these considerations to the case of cork formation we are dealing with the origin of a tissue which arises from the

meristematic activity of parenchyma occupying very different positions in different plants. An analysis of these occurrences of the phellogen is attempted in the succeeding pages and it will be found that in every case good reason can be assigned for the assumption that the phellogen activity had to be preceded by a slow accumulation of sap either from the exudation activity of adjacent parenchyma or more usually from the adjacent vascular strands. The accumulation of the sap can in the majority of cases be traced to a previous blocking of a parenchymatous surface, occurring in close proximity to the region where the phellogen subsequently arises. This blocking, frequently due to deposition of suberin or cutin upon surfaces exposed to air, has often been reported but so far as we are aware its causal connection with the subsequent meristem activity has not previously been emphasised. Our approach to the subject from this point of view has led us to pay special attention to the chronological order in which the various processes associated in cork formation occur.

We are hopeful that the simple causal sequence thus suggested in connection with the process of cork formation may prove of value in interpreting the results of practical experience in such processes as pruning, grafting and the cutting of potato sets before planting. We shall be the more gratified if that hope proves well grounded because the prosecution of the work was only rendered possible through a grant made to the University of Leeds by the Ministry of Agriculture.

In the exposition of our special standpoint in reference to cork formation, it is a simplification to begin with the study of wound cork. Having established by reference to other investigators and by our own further experiments our case for a causal sequence in the case of wound cork formation, a brief discussion will show how the same general chain of causation may apparently be traced in the formation of cork in the cushion left upon leaf-fall and in the normal production of cork upon a perennial stem.

I. THE FORMATION OF WOUND CORK

In one case of great practical importance, the tubers of *Solanum tuberosum* L., many statements are available concerning the processes following upon wounding, and these agree as to the main features. Appel⁽¹⁾ makes it very clear that if the cut surface of the potato is left at room temperature in a moist atmosphere, the first stage in healing is the deposition of an impermeable brown deposit

along walls and intercellular spaces, in an unbroken layer just below the cut surface. Appel emphasises the fact that this impermeable deposit is of great practical significance, because at an average temperature it forms within twelve hours, whilst the periderm formation, the product of a meristem, will only arise within 48 hours and will not be a very effective barrier until somewhat later. The pathogenic organism *Bacillus phytophthorus* (Appel) can penetrate deeply into a cut surface within a few hours: as, however, it fails to penetrate if sown upon the cut surface after twelve hours, it is clear that its entry is prevented by this preliminary brown deposit.

This brown deposit is considered by Appel to be suberin and he confirms the statements of earlier investigators, Kny(14) and Olufsen(18), that the formation of suberin is definitely connected with access of oxygen. Thus he explains its inadequate formation in potato tubers placed after cutting in warm dry air, in that the cut surface then dries rapidly and the entry of air is prevented by the hard dry surface. Suberisation in such cases therefore occurs at irregular depths in small patches around intercellular spaces and not in a continuous layer. As the hard outer layer often cracks irregularly and in any case softens and permits the entry of fungi and bacteria when the tuber is placed in moist earth, the potato sets are very subject to disease when planted. The grafting experiments of Kabus(13) when cut tubers of potato were pressed closely together gave further evidence that the preliminary suberisation was connected with air, as if these cut surfaces made good contact, subsequent staining with Sudan III showed patches of suberin developed only at places where the two surfaces had imprisoned an air bubble.

The access of air to the cut surface may be prevented in various ways; in Kabus' experiments the potatoes were cut under previously boiled water, the cut surfaces pressed together and then bound together by some material to prevent subsequent access of air. Under these conditions, even potatoes from which all buds had been removed would slowly develop cellular outgrowths, of the nature of intumescences, which interpenetrated the two opposed surfaces and finally bound them together, without hindrance from suberisation and without the intervention of a meristem. Similarly both Olufsen(18) and Kabus found that if a piece of potato were removed by a cork borer, then pressed back into the hole and the ring-shaped cut in the outer surface sealed, no suberisation occurred at the cut surface, but the spaces created by the injury were bridged by proliferating cells without the intervention of a meristem. Kabus cut

out a cylinder of the "Magnum bonum" potato, 1.5 cm. in diameter, and placed this in moist air until the exposed cut surface of the cylinder was covered with periderm. He then cut the cylinder across transversely and joined the cut ends together under the protection of a rubber band. He found that the cylinder would grow together again without the activity of a meristem, provided that one of the cut surfaces included at least one tracheid from a vascular strand.

These experiments show that if suberisation does not occur meristem formation may not follow. On the other hand a meristem may form without any previous suberisation of the cut surface. Thus Appel's observation on the surface of tubers dried in the sun showed that very little suberisation occurred here, but such rapidly dried potatoes usually show a slight meristem formation beneath the hard dry surface although it is somewhat irregular. A simple experiment which may easily be performed will also show meristem formation without any suberisation. If the cut surface is immediately covered with melted paraffin wax and left a few days, examination will show regular meristem formation without a trace of previous suberisation. These cases of meristem formation are significant. In every case the essential antecedent to meristem formation is the blocking of the cut surface. When the surface is kept moist and suberisation prevented as in the experiments of Olufsen and Kabus described above, then the cut surface is not blocked and meristem formation does not occur. It will be found also that if the cut surfaces are left under boiled water for a week or two, suberisation will not occur and meristem formation does not follow. This experiment and the comparison with the behaviour of cut surfaces under paraffin wax have been proved sufficiently reliable to use as class experiments.

Meristem formation in these experiments with potato tubers may thus be attributed to the blocking of the cut surface. This blocking then appears to react upon the tissues within, through the accumulation in the walls and intercellular spaces of a sap which provokes the activity of a meristem. In this connection attention may be drawn to the following further facts. Appel in his experiments shows that in the cut potatoes the vascular bundles show a different staining reaction with ammoniacal gentian violet to considerable depths below the cut, a reaction connected by him with the presence of secreted substances in the vascular strand. As a result of the cut, moist air has entered the tissue and probably caused increased turgor followed by rapid excretion. The cells at the base of the bud probably contribute readily to the secretion, hence Kabus finds that

portions of tubers bearing buds most readily grow together by intumescence-like outgrowths. The production of this excretion probably explains Olufsen's observation that the larger the wound the moister it must be kept to enable it to heal, also the prompter rate of healing shown by new potatoes when compared with last year's tubers of the same variety. The use of such an excretion in the necessary cell proliferation may also explain Kabus' observation, cited above, that the two halves of the "Magnum bonum" cylinder only reunited if a tracheid appeared at one cut surface. Haberlandt (7, 8, 9) has also emphasised the dependence of cork formation upon a supply of substances from the vascular strand though he traces these substances to the phloem elements.

The case for the necessity of such an accumulation of sap to initiate the meristem activity is much strengthened if we extend our observations from the case of the potato tuber to the more general phenomena of wound healing. Here again the same two processes may be traced, first the closure of the wounded surface and secondly the appearance of the meristem. As the available data are less complete we will quote chiefly from our own experiments. The first closure of the wound again appears to be due to a deposit of suberin or some allied substance. Kabus found that this substance did not stain with Sudan III: in our experiments the brown deposit resisted concentrated sulphuric acid, went brown with iodine reagents, and frequently stained with Sudan III. We probably have to do, however, with a mixture of substances, in part produced by chemical decomposition in the dying cells at the cut surfaces, in part deposits from the excreted sap which evaporates at the exposed surface.

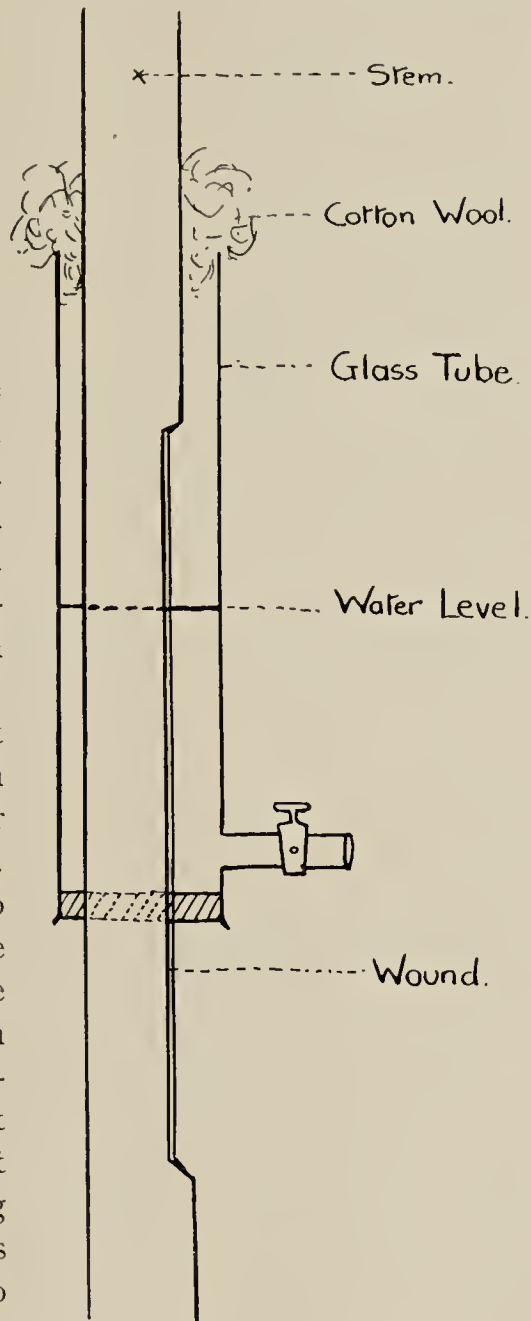
The following experiments explain themselves, in the light of the previous discussion. On March 21st, the epidermis was scraped off a long stretch of the stem of a begonia growing in a greenhouse. The exposed surface was partly covered with melted paraffin wax. On April 25th the two surfaces showed marked differences, the waxed surface being very much less brown. On staining sections with Sudan III the outermost layer of collenchyma only was slightly suberised under the wax, and the five to six layers of cells below this, formed by the phellogen, were quite free from suberin. At the unwaxed surface more layers of cells bore a deposit of suberin, fewer cells had been formed by the phellogen but the outer two or three layers of periderm were strongly suberised. The slight suberisation under the wax may probably be accounted for by the gradual

splitting of the wax surface. It seems evident that with complete exclusion of air no suberisation would occur.

On March 7th cut surfaces of a begonia stem were similarly covered with gelatin. Examined on March 23rd, suberisation had occurred to a considerable extent though perhaps not so freely as at the completely exposed surface. But a gelatin film could not be expected to keep out the air in the same manner as a film of paraffin wax. Meristem formation had begun in both cases. Stems covered with gelatin in this manner on March 7th were re-examined on May 23rd. In this case there was clearly both a larger amount of suberisation and a more active meristem present on the freely exposed wound surface than on the one covered with gelatin.

On March 7th an exposed cut surface was partly covered with distilled water in the manner shown in the diagram (Text-fig.). The water was renewed daily to avoid the development of disease as far as possible. The cut surface was examined on March 19th and more suberisation had occurred on the surface of the cut above the water, only a slight indication of suberisation being obtained in the superficial cells covered by the water. As no meristem had yet formed, the

experiment was repeated, in this case the cut surface extended below the base of the tube as well as above the water in the tube. The experiment was started on March 18th and stopped on April 6th. The cut surface both above and below the water showed much suberin deposited and the presence of a meristem. In the region under



water there was slight suberisation of the outer layer of collenchyma but no trace of a meristem.

The absence of a meristem suggested to us incomplete blocking of the exposed surface in contact with the water. This might be due to the products which accumulated at the surface in the air, diffusing under the experimental conditions into the water. In each experiment the water changed each day was collected and kept. At the end of the experiment it was evaporated down and the residue in the evaporating dish extracted with boiling chloroform. The chloroform extract, filtered and evaporated, left a little amorphous waxy deposit which stained yellow brown with iodine. Treated with iodine and dilute sulphuric acid, part of the substance gave a reddish reaction very reminiscent of phellonic acid (see Priestley⁽²⁰⁾ for an account of the organic acids present in suberin). After saponification with boiling alcoholic potash, the residue from evaporation of the water was again evaporated to dryness and taken up with boiling chloroform. A much larger quantity of the residue now went into solution and this substance after evaporating off the chloroform again gave the phellonic acid reaction. As however the residue in part dissolved in ether, in which phellonic acid is insoluble, it cannot be entirely composed of phellonic acid, and the ether soluble constituent also gave a reddish violet reaction with iodine and sulphuric acid. These reactions suggest then that there had diffused from the injured surface into the water substances which, like the suberogenic acids, were soluble in fatty solvents, and which also were partly present as condensation products, insoluble in fatty solvents without previous saponification. At the surface exposed in air the accumulation of these substances may well be responsible for the stronger and more widely distributed suberin reaction.

In these experiments with begonia we obtain evidence again that periderm formation involves two processes, first a suberisation which seals the injured surface, secondly a meristem formation which follows upon the accumulation of sap at the injured surface. If a plant is taken which unlike begonia has a secondary endodermis in the stem, this will completely retain the sap pressure within it (Priestley and North⁽²²⁾). A superficial wound should then be followed by suberisation without subsequent meristem formation. Experiment shows that facts are in accordance with this expectation. The stem of *Camellia japonica* L. has a strongly developed secondary endodermis, in which all walls are suberised. The young stem has a clear green cortical tissue outside the endodermis. Superficial cuts

were made in this green stem on March 11th, and subsequent examination on April 6th and April 18th showed suberisation of the exposed cells without any phellogen appearing. On the other hand deep cuts or pinpricks penetrating the endodermis were followed in the same time, not only by superficial suberisation but by phellogen production and activity beneath the injured endodermis.

There are certain problems left in the literature which we think can be cleared up in the light of the above facts.

Holden⁽¹²⁾ has studied wound reaction in the Filicinean petiole, practically confining his attention to Leptosporangiate ferns. These ferns develop at an early stage Kroemer's⁽¹⁵⁾ "Secondary" type of endodermis with complete suberisation of at least one tangential as well as the radial wall (Bäsecke⁽³⁾). It is therefore significant that whilst in all cases closure of the wound by processes of the nature of suberisation or wound gum formation was observed, meristem formation was only observed in wounds made at a region where the endodermis would only be partially differentiated or in Kroemer's primary stage. To avoid recurrence to the case of the Filicineae, it may be pointed out here that natural meristems, analogous to periderm formation, are only reported from the Eusporangiate Marattiaceae (Massart⁽¹⁶⁾, *loc. cit.* p. 27) in which the endodermis in the petiole is missing and in the stem is only in the "primary" stage.

Wächter⁽²⁹⁾ has drawn attention to the fact that cuts on the cortical surfaces of the stem of *Hippuris vulgaris* L. are followed by suberisation and secretion of wound gum at the exposed surface, but that traces of meristem are never seen. As the stem of *Hippuris*, like that of most submerged aquatic plants, has a well-marked endodermis, this case is exactly parallel to the wound reaction described above for *Camellia*. This incapacity to form periderm seems general amongst aquatic Phanerogams (see Massart, *loc. cit.* p. 47). The one exception known to us, *Polygonum amphibium* L., is the one submerged aquatic stem in which a superficial cork layer is naturally present and in which cork is formed to heal cortical injuries: it is also the one submerged stem in which no trace of either a primary or secondary endodermis has been found.

Massart also draws attention (*loc. cit.* p. 50) to the incapacity of the cortical cells of roots to form wound cork. He discusses it in relation to the age of the tissues, but the real difference between the behaviour of the young cortical cells of the root of *Vicia Faba* L. and the shoot of *Sambucus nigra* L., contrasted by him, probably lies

in the fact that the endodermal barrier in the young root prevents the accumulation of sap essential to the phellogen (see p. 256).

Schneider-Orelli(27) has made some observations on apple and pear fruits which also admit of explanation from this point of view. The young fruits react to wounds by forming cork tissue, the ripe fruits do not. But this is not merely a question of age because the young fruits removed from the tree no longer form cork when wounded. The conclusion is natural that the cork formation depends upon the sap pressure still active in the young fruit upon the tree and no longer operative when the fruit is removed from the tree or has ripened.

Finally a reconsideration of the phenomena of wound healing in the case of leaves shows that they fall readily into line from this point of view. Blackman and Matthaei(4) have given a general account of the wound reaction in leaves. It may be recalled that all the various modifications of the process described show the following features in common. First a solid mass of tissue is formed at the cut surface by outgrowths from adjacent mesophyll cells, this proceeding *pari-passu* with the dying of the cut cells and some of their neighbours and the suberisation of the walls at this region. Within this solid mass of tissue, which seals off the internal intercellular spaces from the external air, there now develops a meristem in some cases but not in all. Under conditions of considerable moisture this meristem layer may act as an absciss layer and exfoliate tissues bordering on the injury; under drier conditions it forms periderm.

From our present standpoint these phenomena make a natural sequence. The only point we wish to emphasise is that under certain conditions, and in some plants, meristem formation follows the occlusion of the air spaces and sealing of the cut surface. This took place in Blackman and Matthaei's experiments, even in leafy branches cut off from the tree, but kept with their bases in water and their leaves in a moist atmosphere. Under these conditions (Priestley and Armstead(21)) it is quite possible for sap pressure to be developed within the tissue after the closure of the cut base of the branch by suberisation. The subsequent formation of callus, and, in the case of Oleander leaves, of crops of adventitious roots, is evidence that such sap pressure had indeed developed and therefore meristem formation was possible.

To obtain further evidence we have carried out experiments with injured leaves of *Prunus Laurocerasus* L., and *Camellia japonica* L., in which the cut leaves have been supplied with water, either by

standing them in water or applying it under increased or reduced pressure by means of a mercury column. The pressures were small, averaging about ten centimetres of mercury. The differences were very striking; under even this small reduced pressure these leaves were soon drooping and dry and even the young leaves ceased to grow. These leaves had to be left out of the comparison, but the leaves under increased pressure compared with those standing in water showed striking differences within the short period April 29th to May 12th. Under pressure a sharp brown edge had formed around the cut in one day, whilst without the pressure the brown edge formed much more slowly. By May 12th the cuts in the leaves under pressure could be distinguished with the naked eye by the thin translucent line, due to the formation of a meristem bordering the clearly defined brown margin on all sides. In the leaves not under pressure the brown margin was wider and lighter and there was still no meristem. In young leaves under pressure meristem formation was clearly visible in sections by May 10th. The interesting exfoliation patterns described by Blackman and Matthaei in the leaves of *Prunus Laurocerasus* kept in a moist atmosphere, seem to receive considerable elucidation if it is considered that the formation of the meristem, functioning here as an absciss layer, depends upon a sap pressure, the incidence of which throughout the leaf is determined by the venation system.

II. LEAF FALL

The formation of periderm at the leaf scar requires only brief consideration for which the necessary data are provided by Tison's (28) very comprehensive memoir on the subject. Reference to this paper will show that at the time the leaf falls, as the result of various types of activity in an absciss layer, the cushion of cells at the surface of the scar has already undergone, or immediately undergoes, a process of lignification and suberisation, the lignification being specially strong in the deeper seated layers. When the tissues of the leaf scar are blocked in this manner, the vascular strands being blocked by tylosis or the deposit of wound gum or other substance within the vessels and tracheids, then the formation of periderm may occur within the cushion, below the suberised and lignified surface layers. In no case does the meristem arise before the tissues of the scar are sealed by the previous suberisation. On the contrary in many cases no phellogen appears until the sap rises in the following spring; in the relatively few cases where the meristem is formed in

the same season as the leaf falls, Tison's data suggests that the suberised cap of tissue is formed unusually early so that the leaf scar is blocked before the sap pressure has ceased to be a significant internal factor with the advent of the autumn.

One small point of difficulty arises from a consideration of Tison's observations upon those compound leaves in which absciss layers are formed not only at the base of the main petiole but also at the base of the leaflets. In two cases, *Juglans regia* L. and *Pyrus aucuparia* L., he describes a periderm, very slight in character, as forming at the base of the leaflet scars, in the tissue of the petiole *after* its fall from the stem. This phenomenon was unexpected, but it is a point to which it is hoped to direct further attention as opportunity offers. So far, in the fallen petioles collected in this country, no trace of such a periderm has been found, but in *Fraxinus excelsior* L. a cork meristem has been found at the base of leaflets which have fallen before the main petiole. Probably closer examination will show that all cork layers at the base of leaflets have arisen whilst the meristem was still able to receive supplies from a petiole still in communication with the stem.

III. NORMAL CORK AND LENTICEL FORMATION

In the light of the previous discussion of wound cork and of the periderm arising below a leaf-scar the problem of normal cork formation upon stem and root may be visualised from an unusual angle. In any species the position of the cork phellogen seems quite definite but in different species this meristem arises in very different positions: it may be nearly superficial or even arise in the epidermis or it may be as deep seated as the pericycle. This question of the position in which the periderm arises obviously requires analysis to see whether it admits of correlation with a supply of sap from the vascular tissue and a blocked surface external to the phellogen which provides for an accumulation of this sap.

In the root the phellogen may occur in one of two alternative positions, either just within the endodermis (the usual position—de Bary(2), *loc. cit.* p. 553) or just within the exodermis. A very extensive analysis of cases will be required before any generalisation can be advanced with confidence to cover all cases of periderm formation in the root, but the following tentative conclusions have been found to cover all cases so far studied. When the growing root is young, the endodermis is in the primary stage (Priestley and North(22)) with fatty substances deposited in the radial and trans-

verse walls and with the protoplast firmly attached right across the cell to this girdle, the Casparian strip. As this fat impregnated layer is very impermeable, and at this stage the protoplasts are also relatively impermeable the endodermis provides a very complete barrier to the outward leakage of organic solutes. If a cork layer is now laid down the phellogen becomes active within the endodermis and the cork is therefore pericyclic in origin.

As the root grows older, the protoplasts of the endodermis become more permeable, but this alteration is usually associated with an accumulation of fatty substances all over their surface and the consequent formation of a suberin lamella. The cells of the endodermis have then passed into the secondary stage (Priestley and North(22)). If all the endodermal cells pass over into this stage then the endodermis has become a still more effective barrier, preventing the diffusion of inorganic salts as well as organic solutes. Within such a secondary endodermis a cork layer frequently arises in the pericycle, but no case has ever been seen of a phellogen active in the cortex outside such an endodermis.

On the other hand the fat deposits sometimes fail to accumulate in certain passage cells which therefore remain uncoated with a suberin lamella. The protoplasts of these cells are now readily permeable to organic solutes, as may be shown by the ease with which they admit the entry of organic dyes, and such an endodermis permits the formation of a cork phellogen external to it in the cortex. When such a phellogen arises, it always appears just below the exodermis which may be expected to act as the necessary blocking surface causing the accumulation of the sap flowing from the vascular tissues. An example of such a cortical cork layer arising outside an incomplete secondary endodermis and beneath a sclerenchymatous exodermal region is provided by the aerial root of *Philodendron erubescens*. *Monstera deliciosa* Liebm. provides an example of an aerial root which as it grows older never produces a secondary endodermis. As the protoplasts of the primary endodermis become more permeable, cork appears below the exodermis. In a root of *Monstera*, 20 feet long, obtained from the Cambridge Botanic Garden, a primary endodermis appeared within a few (8) inches of the tip; 27 inches from the tip a cork phellogen was visible and two or three layers of cells had been formed by its activity; at the base of the root six to eight layers of cork cells were present and a thick band of sclerenchyma had also formed outside the endodermis, but this endodermis itself remained primary.

One further case deserves mention. Mylius(17) has described a polyderm formation which is practically a type of multiple secondary endodermis. Within the original secondary endodermis a phellogen becomes active and cuts off two or three rows of cells. One of these rows then becomes converted into a secondary endodermis by the deposit of a suberin lamella upon every cell, the meristem then becomes active within this and the process may be repeated many times. A magnificent example of such a polyderm is provided by the raspberry, *Rubus Idaeus* L.

In the stem, Douliot(6) shows that the cork may be superficial, pericyclic or intermediate in origin. In view of the very numerous cases to be examined a causal explanation of the position taken by the phellogen can only be very tentative in nature. The following observations seem, however, to be relevant. In all the cases of pericyclic cork so far examined, with one exception, the stems have been found to possess a complete secondary endodermis within which the phellogen arises. Examples are provided by most dicotyledons growing upon British peat (Priestley and Hinchliff(25)), by *Escalonia rubra*, *Saxifraga rotundifolia* and *Camellia japonica*. Many underground rhizomes, as for instance several species of Geum, possess a secondary endodermis, within which a cork layer arises, the activity of this phellogen often leading later to the exfoliation of the cortex. All these cases are obviously in line with the general hypothesis put forward in this paper.

The exception is provided by species of *Ribes* in which no functional endodermal barrier can be found outside the deep seated phellogen. But experiment in which dyes such as acid green were forced under pressure into these stems showed that these dyes failed to leak into the outer cortex, save in the region of the nodes, even before any cork barrier prevented their diffusion. Further examination led to the conclusion that the superficial layers of the stem of these plants were really petiolar in nature (compare Saunders(26)) and save at the nodes were still delimited from the inner regions of the stem by the presence of a cuticle which could be traced by careful staining with Sudan III. The phellogen in this plant is not pericyclic in origin and its apparently deep seated origin is due to the presence of this envelope of petiolar tissue upon the stem.

The case of stems without a functional endodermis now requires consideration. In these stems it is a very suggestive fact, well established by the researches of Devaux(5), that periderm formation

begins at the lenticel, before the phellogen forms a continuous layer encircling the cortex. Devaux's extensive investigations provide other data of considerable interest, notably that the number of lenticels developed per internode suggest that lenticel formation is in direct relation to the vigour of growth of the shoot. As a result of his experimental analysis of the external and internal conditions leading to the formation of what he terms the primary lenticels upon a shoot, Devaux arrives at the conception that the formation of the lenticel depends upon an adequate supply of internal moisture, the "hydrose" of the tissue. As he also develops the idea that the continued functioning of the lenticel consists of an alternate closing, owing to extensive suberisation, and opening owing to the internal meristematic activity forcing the tissue through the hermetically sealed surface, an alternation of "cicatrization" due to dryness of external and internal conditions and of proliferation due to excess of internal moisture, it is obvious that in one respect Devaux is very close to the point of view developed in this paper.

He departs from it widely however in that he sees the cicatrization of the lenticel beginning with the proliferation of the subepidermal cells lining the stomatal chamber. He notes an early cutinisation of the surface of these cells lining the intercellular spaces bordering on the stoma (Devaux, *loc. cit.* pp. 81 and 97) but he regards this as a secondary feature. We should anticipate on the other hand that in this blocking of the surface cells bordering upon the stomatal chamber we have the causal feature preceding the sap accumulation, which in its turn brings about proliferation and the meristematic activity which results in the formation of the lenticel.

Attention was therefore specially directed to this point, and upon examining sections of young internodes of the stems of cork-forming plants clear evidence was obtained of the early cutinisation of the exposed surfaces of the cells bordering upon the stomatal chamber. In *Pyrus torminalis* Ehrb., *Acer pseudoplatanus* L. the first internode, in *Sambucus nigra* the second and in *Syringa vulgaris* L. the third, showed the clear red stain of a fatty deposit all round the surface of the stomatal cavity when sections were examined in Sudan III.

It seems that when the endodermis is not functional the positive pressure of sap within the vascular strand will be effective in irrigating the tissues right up to the subepidermal layer bordering upon the stoma which is probably thus supplied with water and solutes upon its free surface. As evaporation proceeds from this surface the

solute will be left deposited along the surface exposed to the air and thus a continuous deposit, very similar apparently in its nature to the original cuticle, appears upon the exposed surface. The presence of fatty substances in this layer is not surprising in view of the fact that Priestley and Armstead(21) found an ether soluble fatty substance present in the sap rising from the vine root, whilst Hansteen-Cranner(10, 11) has shown that fatty substances, both water soluble and water insoluble in nature, seem to form an invariable constituent of the walls in parenchymatous tissues. If these deposits at the wall surface are to be associated with such a drive of sap from the vascular strands, then the sub-stomatal cavity may be free from them where the stem endodermis is functional. Examination of the stomata upon the green cortical region of the stem of *Camellia japonica* certainly failed to show any signs of such deposits, stainable in Sudan III, the contrast in comparison with the stomatal cavities of the plants previously mentioned being very striking.

Douliot(6), in his extensive study of the distribution of the periderm in Dicotyledonous stems, draws attention to the marked effect of light upon the formation of periderm in the stem. In certain cases, *Prunus spinosa* L., *Acer oblongus* and *Virgilia lutea*, no periderm at all had formed upon the shady side of the stem when active periderm formation had occurred upon the sunny side. Some unpublished work by Miss R. Rea, M.Sc., in this laboratory, has drawn attention to the rôle played by sunlight in causing the rapid condensation of phellonic acid, one of the suberogenic acids (see Priestley(20)), but we are inclined to attribute this distribution of cork under lateral illumination to the more rapid evaporation from stomata in the sun and thus the earlier blocking of the substomatal apertures.

The facts and observations quoted above lead to the conclusion that the natural formation of cork at the surface of the stem follows as the result of the same general causal factors, previous blocking of sap flow, either at the endodermis or at the sub-stomatal region, followed by accumulation of sap to be followed again by the appearance of a meristematic phellogen.

SUMMARY

I. Cork formation has been studied from a generalised standpoint arising out of a study of the effect of the sap contained within the vascular strand upon the meristematic activity of the tissue supplied by these strands.

2. Wound cork formation, the scar left at leaf fall and natural cork formation all show the following causal sequence: first, the blocking of a parenchymatous surface, usually by a deposit of suberin or cutin formed in presence of air; secondly, the accumulation of sap at the parenchymatous surface thus blocked; and thirdly, the consequent development and activity of a phellogen amidst this parenchyma.

3. The pericyclic origin of a periderm must be attributed to the presence of a functional endodermal barrier when the phellogen is formed.

4. Special cases, such as the general absence of periderm formation in the cortex of roots, in the axes of aquatic plants, and in leptosporangiate ferns, provide additional evidence for the correctness of the causal sequence stated above.

5. In the case of cork formation in leaves experimental evidence is supplied to show that the meristem formation depends upon a sap pressure within the tissue.

6. Further experimental evidence is also supplied, confirming previous statements to the effect that the preliminary suberisation depends primarily upon air.

7. It is further shown that if the parenchymatous surfaces are artificially blocked by experimental methods, a subsequent sap pressure may produce meristematic activity as a result of which tissues like normal periderm may be formed, save that the walls are not suberised.

REFERENCES

- (1) APPEL, OTTO. Zur Kenntnis des Wundverschlusses bei den Kartoffeln. *Ber. der deutsch. Bot. Ges.* 24, pp. 118-122. 1906.
- (2) BARY, DE. *Comparative Anatomy of Phanerogams and Ferns*. Oxford, 1884.
- (3) BÄSECKE, PAUL. Beiträge zur Kenntnis der physiologischen Scheiden der Achsen und Wedel der Filicinae. *Bot. Zeitung*, 66, p. 25. 1908.
- (4) BLACKMAN, F. F., and MATTHAEI, GABRIELLE L. C. On the reaction of Leaves to Traumatic Stimulation. *Annals of Botany*, 15, pp. 535-546. 1901.
- (5) DEVAUX, H. Recherches sur les Lenticelles. *Ann. des Sci. Nat.* VIII^e Sér. 12, pp. 1-240. 1900.
- (6) DOULIOT, H. Recherches sur le Périderme. *Ann. des Sci. Nat.* VII^e Sér. 10, pp. 325-395. 1889.
- (7) HABERLANDT, G. Zur Physiologie der Zellteilung. I. *Sitzungsber. der Königl. Preuss. Akad. der Wissensch. Berlin*, pp. 318-345. 1913.
- (8) ——— Zur Physiologie der Zellteilung. II. *Sitzungsber. der Königl. Preuss. Akad. der Wissensch.* pp. 1096-1111. 1914.
- (9) ——— Zur Physiologie der Zellteilung. III. *Sitzungsber. d. K. Preuss. Akad. der Wissensch.* pp. 221-234. 1921.

- (10) HANSTEEN-CRANNER, B. Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III. Beiträge zur Biochemie und Physiologie der Zellwand lebender Zellen. *Jahrb. wiss. Bot.* **53**, pp. 536-598. 1914.
- (11) ——— Beiträge zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. *Ber. der deutsch. Bot. Ges.* **37**, pp. 380-391. 1919.
- (12) HOLDEN, H. S. Some Wound Reactions in Filicinean Petioles. *Annals of Botany*, **26**, pp. 777-793. 1912.
- (13) KABUS, BRUNO. Neue Untersuchungen über Regenerationsvorgänge bei Pflanzen. *Beitr. zur Biol. der Pflanzen*, **11**, pp. 1-52. 1912.
- (14) KNY, L. *Ber. der deutsch. Bot. Ges.* **7**, pp. 154-168. 1889.
- (15) KROEMER, K. Wurzelhaut, Hypodermis und Endodermis der Angiospermenwurzel. *Bibl. Botanica*, Heft 59, pp. 1-143. 1903.
- (16) MASSART, JEAN. La Cicatrisation chez les Végétaux. *Mém. de l'Académie Royale de Belgique*, **57**, pp. 1-68. 1898.
- (17) MYLIUS, G. Das Polyderm. *Bibl. Botanica*, **18**, Heft 79. 1913.
- (18) OLUFSEN, L. Untersuchungen über Wundperidermbildung an Kartoffelknollen. *Beih. zum Bot. Centr.* **15**, pp. 269-308. 1903.
- (19) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytologist*, **19**, pp. 189-200. 1920. And **21**, pp. 41-47. 1922.
- (20) ——— Suberin and Cutin. *New Phytologist*, **20**, pp. 17-29. 1921.
- (21) PRIESTLEY, J. H. and ARMSTEAD, DOROTHY. The Physiological Relation of the Surrounding Tissue to the Xylem and its Contents. *New Phytologist*, **21**, pp. 62-80. 1922.
- (22) PRIESTLEY, J. H. and NORTH, EDITH E. The Physiological Anatomy of the Endodermis. *New Phytologist*, **21**, pp. 113-139. 1922.
- (23) PRIESTLEY, J. H. and TUPPER-CAREY, R. M. The Water Relations of the Plant Growing Point. *New Phytologist*, **21**, pp. 210-229. 1922.
- (24) PRIESTLEY, J. H. and EVERSLED, A. F. C. H. A Quantitative Study of the Growth of Roots. *Annals of Botany*, **36**, pp. 225-237. 1922.
- (25) PRIESTLEY, J. H. and HINCHLIFF, MILDRED. The Physiological Anatomy of the Vascular Plants characteristic of Peat. *The Naturalist*, pp. 263-268. 1922.
- (26) SAUNDERS, EDITH R. The Leaf-skin Theory of the Stem. *Annals of Botany*, **36**, pp. 135-166. 1922.
- (27) SCHNEIDER-ORELLI, O. Versuche über Wundung und Wundverschluss an Pflanzenorganen. *Centr. Bakt.* 2 Abt. **30**, pp. 420-429. 1911.
- (28) TISON, ADRIEN. Recherches sur la Chute des Feuilles chez les Dicotylédones. *Mémoires Soc. Linn. Normandie*, **20**, Sér. 2^e, 4, p. 121. 1900-1901.
- (29) WÄCHTER, W. Wundverschluss bei *Hippuris vulgaris* L. *Beih. zum Bot. Centr.* **18**, p. 1. 1905.

THE STRUCTURE AND FUNCTION OF THE ENDODERMIS IN THE LEAVES OF THE ABIETINEÆ¹

By ISABEL SOAR, Ph.D.

(With 12 figures in the text)

INTRODUCTION

THIS work on the endodermis in the Abietineæ was undertaken with a view to ascertaining, as far as possible, the structure and distribution of the endodermal cells. The leaves are all markedly xeromorphic in character, and an endodermal layer is always present. This state of affairs is in marked contrast to that which obtains in the leaves of most angiosperms where, as a rule, no definite endodermis can be distinguished.

The facts observed suggest that the peculiar structure of the endodermis in the leaves of these gymnosperms is one of the factors which serve to retard transpiration. If this be so, it must be taken into account when considering the xeromorphy of the leaf as a whole, equally with such factors as the number and position of the stomata, the amount of sclerotic hypodermal and epidermal tissue, the reduction of exposed leaf surface and the absence of external dorsiventrality.

METHODS

The following reagents were found to be most useful for determining the constituents of the cell wall in the endodermis. By their use distinction was made between suberin, lignocellulose and cellulose.

Both microtome and hand sections were used.

I. *Iodine and sulphuric acid.*

The cellulose walls swell, become bright blue in colour and are finally dissolved. Lignocellulose layers are stained deep brown, and suberised walls yellow-brown.

¹ Thesis approved for the degree of Doctor of Philosophy in the University of London.

II. *Concentrated sulphuric acid.*

The cellulose and lignocellulose walls swell, the former rapidly dissolving in the acid, whereas the latter carbonise and dissolve much more slowly. Suberised and cuticularised layers do not swell when in contact with the acid and are not carbonised by prolonged treatment with it.

III. *Dilute sulphuric acid.*

This was found to be very useful when used as a clearing agent. By mounting sections in it the presence of pits in the walls of the cells could be demonstrated clearly.

IV. *Phloroglucin and hydrochloric acid.*

If a solution of phloroglucin is allowed to act on the sections for about two minutes, and the sections are subsequently mounted in strong hydrochloric acid, cellulose walls remain unstained, whereas lignocellulose walls are stained bright red and suberised walls are stained a deep pink.

V. *Eau de Javelle.*

By treating sections for twenty-four hours with eau de Javelle the nature of the lignified walls is changed, these acquiring the reactions of cellulose. Thus subsequent treatment with phloroglucin and hydrochloric acid produces no coloration in these walls. Suberised and cuticularised membranes remain unchanged by this treatment, and on the addition of phloroglucin and hydrochloric acid a pink colour is produced where these are present.

If, after the section has been treated with eau de Javelle for twenty-four hours, concentrated sulphuric acid is placed upon it, both cellulose and lignocellulose membranes are rapidly dissolved without carbonisation occurring in the latter, and only the suberised and cuticularised layers of the walls remain.

VI. *Alcoholic solution of Sudan III.*

Sections show a red colour in suberised and cuticularised membranes only.

VII. *Alcoholic solution of alkannin.*

If this is allowed to act on sections for about two hours a red coloration is produced in suberised and cuticularised membranes only.

VIII. *Fresh strong alcoholic solution of chlorophyll.*

Sections immersed in this solution for about half-an-hour in the dark show a green coloration in the suberised and cuticularised layers. This was not found to be such a good differential stain as either alkannin or Sudan III as the green colour does not show up against the yellowish background of the leaf so sharply as does the red.

IX. *Aqueous solution of caustic potash.*

Cuticularised layers appear a bright yellow colour, suberised membranes assume a bright reddish-yellow colour, and lignified walls are stained dull brown. The differentiation is intensified by warming the sections in potash, and prolonged heat causes the suberised and cuticularised layers to decompose and dissolve.

X. *Schultze's solution.*

Cellulose membranes swell and turn blue, lignocellulose walls stain deep brown, and suberised and cuticularised layers yellow-brown.

Useful stains for the differentiation of the walls were found to be:

I. Alcoholic safranin followed by light green in clove oil.

II. Alcoholic malachite green followed by eosin in clove oil.

The text-figures were made from camera lucida drawings and are slightly diagrammatic in character.

RESULTS

The Abietineæ may be considered to include two main groups:

(1) The Pineæ comprising the genera *Pinus*, *Picea*, *Pseudotsuga* and *Larix*.

(2) The Abietæ comprising the genera *Abies*, *Cedrus*, *Tsuga* and *Pseudolarix*.

One example of every genus in each of the two groups was examined, and in the case of *Pinus* several species were dealt with. The following were among those investigated:

I. *Pinus sylvestris* L.

An endodermis is present in both the very young stem and the very young root of the pine seedling, but is absent from all parts of the older plant with the exception of the leaves. In the latter, however, it is always present, its nature varying both with the type of leaf and with its age.

(a) The cotyledons.

Six or seven of these are present and each is triangular in section. Stomata are found on the two radial faces only. There is no double hypodermal layer. The endodermis (Fig. 1) is present as an uninterrupted sheath of rather large and fairly regular cells separating the conducting tissue from the mesophyll. Scarcely any infolding of the walls of the latter occurs and resin canals are absent. All the walls of the endodermis consist entirely of cellulose. Developing tracheidal cells are visible in the pericycle.

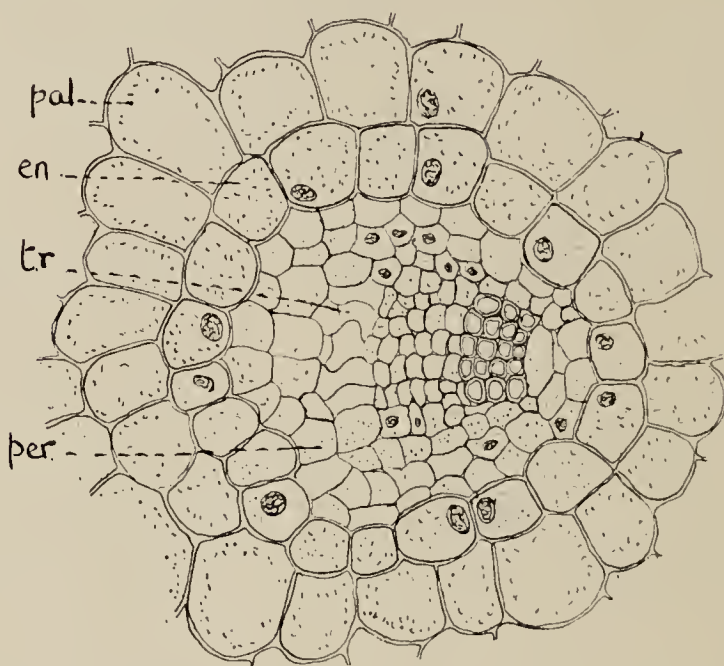


Fig. 1. *Pinus sylvestris* L. Transverse section of cotyledon showing unthickened endodermis. *en.*, endodermis; *pal.*, palisade tissue; *per.*, pericycle; *tr.*, developing tracheidal tissue. ($\times 240$.)

(b) The primary leaves.

These are distinguished from the fascicular leaves produced later by the fact that they grow singly on the stem. They are elliptical in section and bear stomata on both sides. The mesophyll is plicate and two resin canals are present, but there is no double hypodermal layer. The bundle is separated into two symmetrical portions by a large medullary ray. The endodermis (Fig. 2, *a*) appears as an unbroken sheath. The radial and transverse walls are thinly suberised. After prolonged treatment with concentrated sulphuric acid a slight amount of carbonisation is seen in the centre of these walls, and also in parts of the outer tangential walls, showing that a small amount of ligno-

cellulose is present. There is considerable development of transfusion tracheids in the leaf.

(c) The fascicular leaves.

These are semi-elliptical in section, growing in pairs in a sheathing investment of membranous scales. Stomata are present on both sides of the leaf. The epidermis is cuticularised and a lignified hypodermal

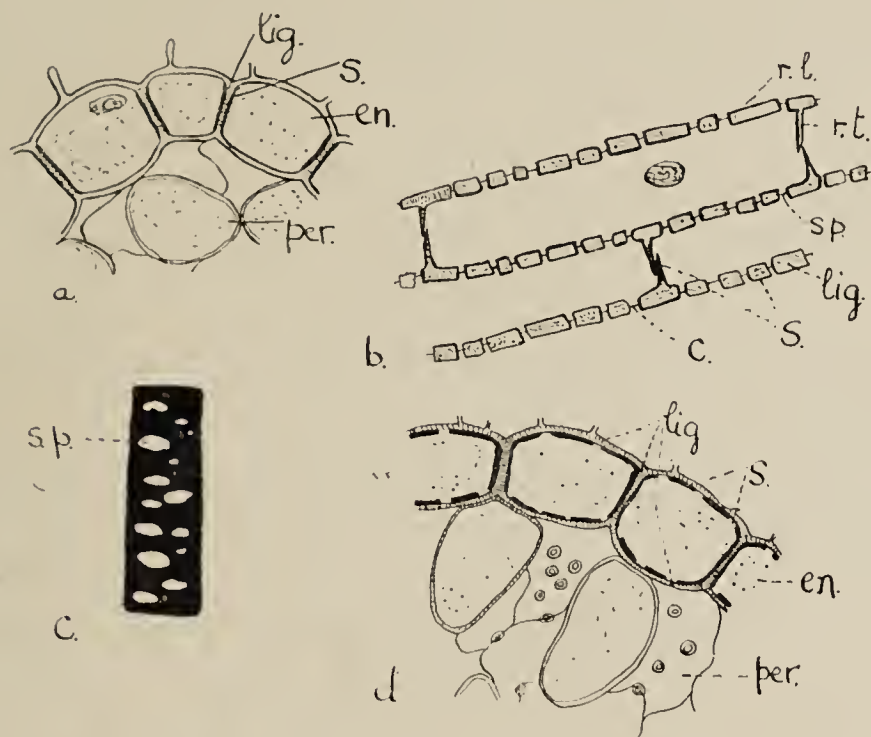


Fig. 2. *Pinus sylvestris* L. (a) Transverse section of primary leaf. Radial walls of endodermis lignified in the middle and suberised at the edges. (b) The endodermal cells of a mature leaf as seen in tangential longitudinal section after treatment with eau de Javelle followed by phloroglucin and hydrochloric acid. Both radial and transverse walls consist of a lignocellulose core enclosed by a layer of suberin. The radial walls are interrupted by cellulose pits. (c) A piece of the radial wall stained with phloroglucin and mounted in hydrochloric acid. The wall is shown in surface view. Simple unthickened pits are present. (d) The endodermis of an old leaf in transverse section after treatment with eau de Javelle followed by phloroglucin and hydrochloric acid. The distribution of suberin on the radial and tangential walls is shown. *en.*, endodermis; *lig.*, lignocellulose; *s.*, suberin; *r.l.*, radial wall; *r.t.*, transverse walls; *s.p.*, simple pit; *c.*, cellulose; *per.*, pericycle. ($\times 240$.)

layer is present which is doubled along almost the whole of its length in old leaves. The adult leaf shows a well-developed regular endodermal sheath, the cells of which (Fig. 2, *d*) have all their walls thickened, the radial walls being suberised and the tangential walls lignified. The tracheidal tissue shows bordered pits which usually appear in

section as a thin layer surrounded by a lignified wall on either side. In very few cases were the pits cut in such a direction as to exhibit the break in the lignified wall. The radial walls were found to consist of a central core of lignocellulose with a thin layer of suberin on either side. The presence of this thin suberised layer can be demonstrated by staining with Sudan III or alkannin, when it takes a deep red colour; or by soaking the sections in eau de Javelle for twenty-four hours, adding phloroglucin, and mounting in hydrochloric acid, when the main central part of the radial longitudinal wall appears yellow whilst a pink line is produced on either side of the cut surface of the wall owing to the presence of the thin suberised layer (Fig. 2, *b*). The central core of the wall is strongly carbonised by sulphuric acid and does not stain with such reagents as alkannin and Sudan III. The suberised layer is generally continued round the corners of the cells towards the tangential walls, particularly so in the case of the outer tangential wall. Numerous simple pits are present in the radial walls (Fig. 2, *c*). The pit membrane is of cellulose.

The tangential walls consist largely of lignocellulose, but both inner and outer walls have deposits of suberin on their inner sides, though these are not continuous over the whole surface (Fig. 2, *d*), patches of unsuberised wall being left here and there. Pits on the inner tangential walls can be seen clearly if the sections are treated with eau de Javelle followed by dilute sulphuric acid. The pits are simple ones communicating with the parenchymatous cells of the pericycle. The latter in their turn are possessed of simple pits where they abut on other parenchymatous pericyclic cells, but pits are absent where they abut on tracheidal tissue (Fig. 3). The transfusion tracheids alone have bordered pits.

The transverse walls of the cell are unpitted and resemble the radial walls in structure, though they are thinner than these. A central core of lignocellulose is present, covered on either side with a thin layer of suberin. Along a central line running across the wall in a radial direction the wall appears thin and it readily breaks here in sections of the leaf (Fig. 2, *b*).

Thus, in the endodermis of the fascicular leaves of *Pinus sylvestris* the radial and transverse walls are completely lignified and suberised, while the tangential walls are lignified and suberised in patches only. In no case were any of the endodermal cells in the adult leaf found to consist of unchanged cellulose, and no passage cells were observed.

The action of alkannin and of Sudan III shows that the tracheidal tissue is suberised to a certain extent, and this is further borne out

by the fact that some of the cells are left undissolved after treatment with concentrated sulphuric acid.

The endodermal cells are all rich in protoplasmic contents and contain numerous starch grains and large nuclei.

(d) Young leaves at the growing point of the shoot.

In sections of young leaves the endodermis appears as a regular layer, the cells of which are full of contents and contain especially large nuclei, the walls being all composed of unthickened cellulose. No infoldings of the mesophyll walls can be seen (Fig. 4, a).

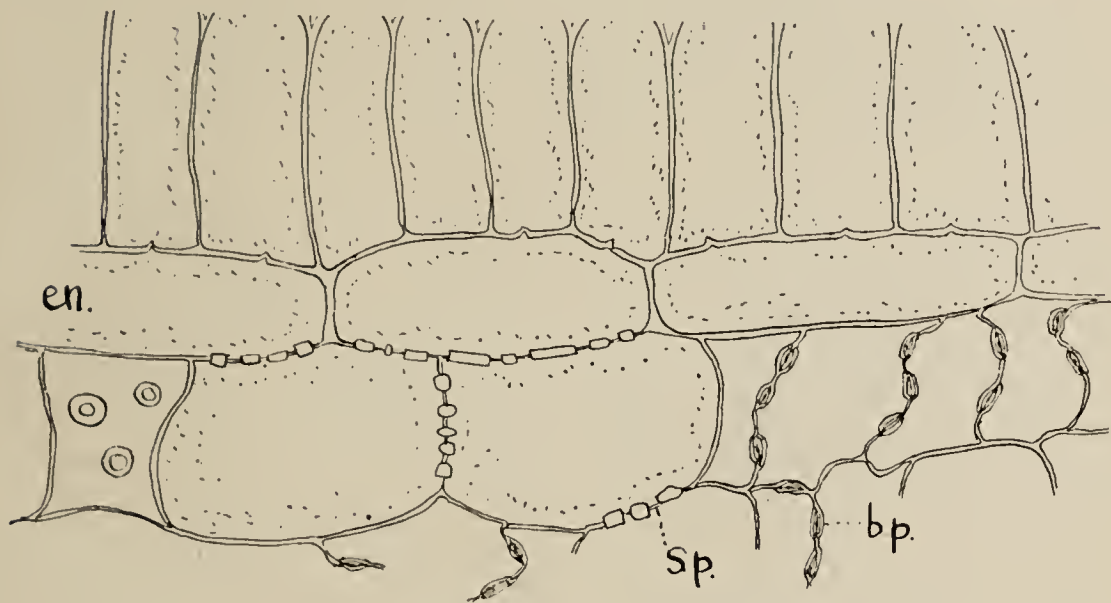


Fig. 3. *Pinus sylvestris* L. Radial longitudinal section of leaf treated with eau de Javelle followed by dilute sulphuric acid. Simple pits (s.p.) are shown in the inner tangential wall of the endodermis (en.) and bordered pits (b.p.) in the tracheidal pericycle tissue. ($\times 240$.)

As the leaf grows older and the characteristic plicate arrangement of the mesophyll appears, each radial longitudinal wall of the endodermal cell becomes thickened with a band of lignocellulose extending over both surfaces of the wall and round the corners on to the tangential walls for a short distance. The tangential walls are unligified and the transverse walls resemble the radial walls in structure, though the latter only are pitted. No sign of the usual wavy appearance due to Caspary's band can be seen in the young radial walls, these being lignified over the whole surface and pitted at frequent and irregular intervals.

At a later stage the radial walls become lignified throughout their thickness, the change appearing first in their outer corners. At the same time a thin layer of suberin is deposited over the surface

(Fig. 4, *b*). The same process occurs in the transverse walls. Finally, the tangential walls become lignified, the inner undergoing this change first (Fig. 4, *c* and *d*). In older leaves irregular deposits of suberin can be found on the tangential walls, the typical structure of the adult form of the fascicular leaf thus being attained.

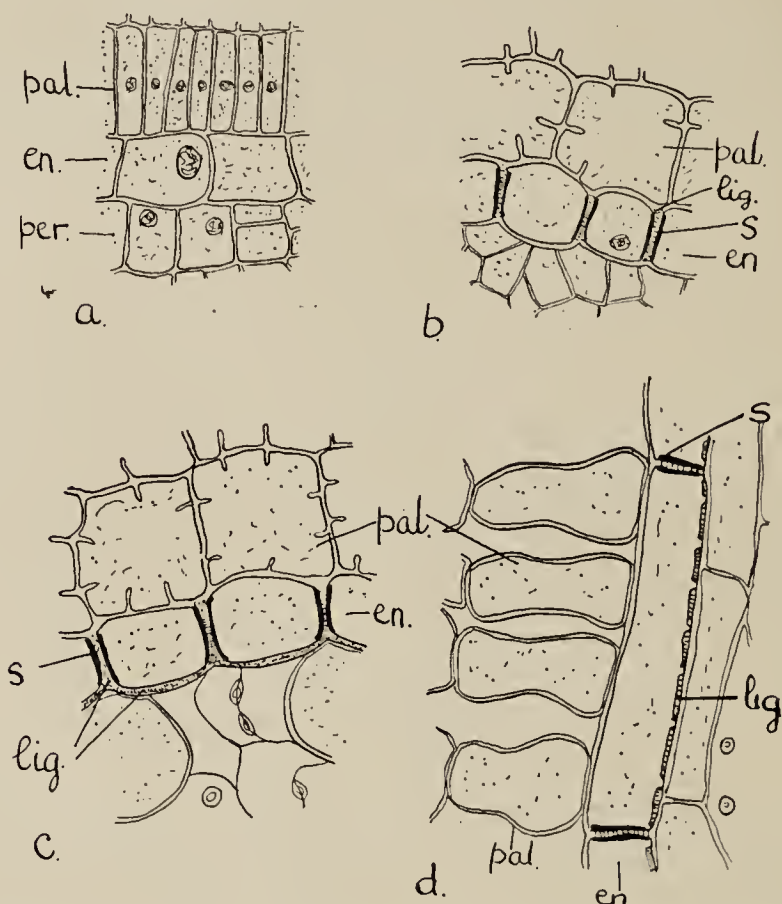


Fig. 4. *Pinus sylvestris* L. (a) Transverse section of young leaf near the base. Endodermis regular but unthickened. (b) Transverse section of fairly young leaf. Radial walls only thickened. They consist of a lignocellulose core with a surface layer of suberin. (c) Transverse section of older leaf. Radial walls lignified and suberised; inner tangential walls lignified. (d) Radial longitudinal section of older leaf. Transverse walls lignified and suberised; inner tangential walls lignified. *en.*, endodermis; *pal.*, palisade tissue; *per.*, pericycle; *s.*, suberin; *lig.* lignocellulose. ($\times 240$.)

(e) The appearance of the endodermis as seen in passing from the dwarf shoot to the leaves.

The evolution of the endodermis at the base of the leaves is shown by the series of diagrams in Text-fig. 5. No trace of an endodermal sheath is visible in the dwarf shoot. Towards the apex of this shoot a concentration of the cortical tissues occurs round the conducting cylinder, the cells becoming smaller and more densely packed.

At the same time the vascular tissue, within which are numerous medullary rays, undergoes concentration, many of the rays disappearing. There is, at this stage, a central pith consisting of thick-walled cellulose elements (Fig. 5, *a*).

The next stage in development consists in the separation, in a radial direction, of the xylem into two groups, thus defining the

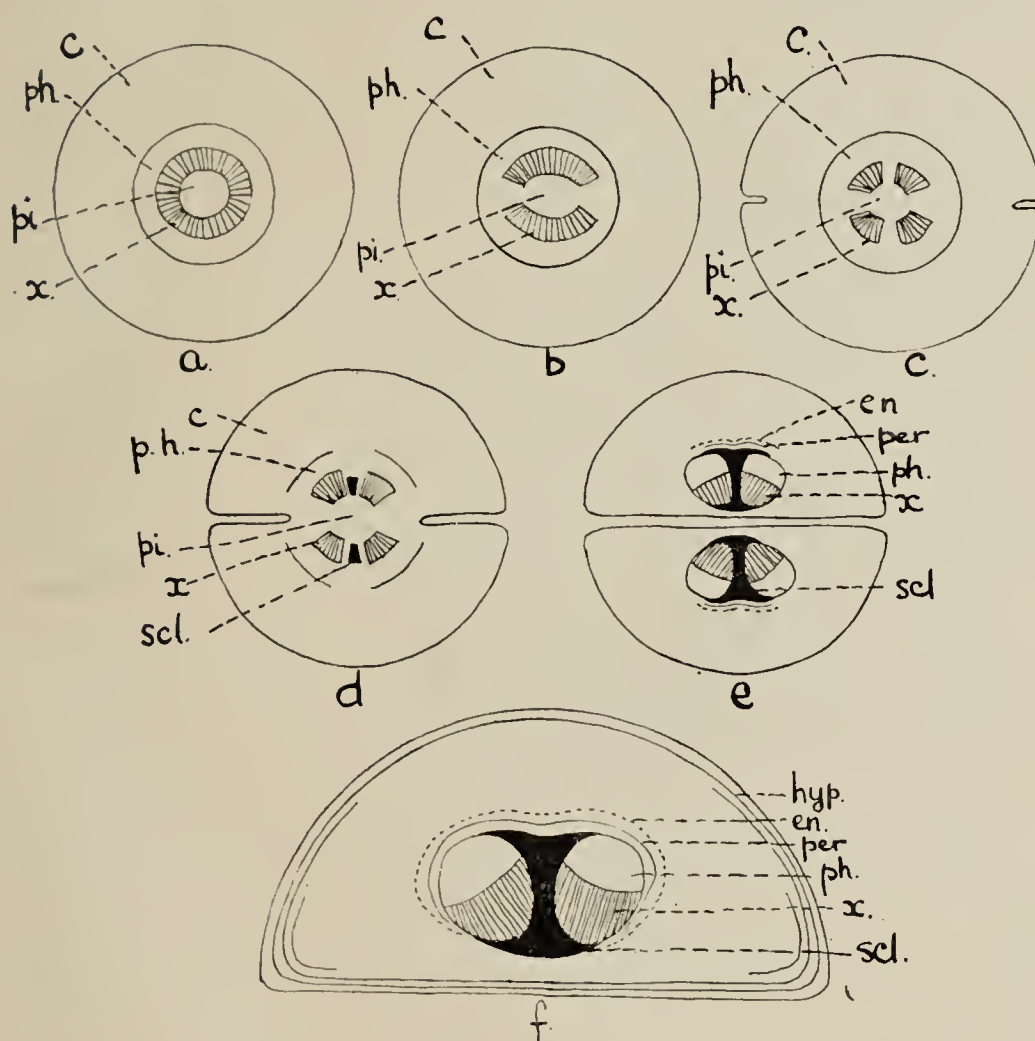


Fig. 5. *Pinus sylvestris* L. (a)—(f). Diagrams showing the evolution of the endodermis of the leaf from the dwarf shoot. *x.*, xylem; *ph.*, phloem; *c.*, cortex; *pi.*, pith; *scl.*, sclerenchyma; *en.*, endodermis; *per.*, pericycle; *hyp.*, hypoderm.

future plane of division of the two needles, which grow laterally on the dwarf shoot (Fig. 5, *b*). Subsequently indentation of the cortex in this plane begins, together with a further separation of the parts of the xylem on each side into two groups corresponding with the two vascular bundles of each leaf (Fig. 5, *c*).

About this stage lignified sclerenchymatous elements appear between the two bundles of each of the needles (Fig. 5, *d*). These elements increase in number, spreading towards the centre of the stem, and finally occupy the entire space between the two vascular bundles and extend round these on either side. At the same time cleavage into two proceeds, the tissues of the leaves becoming more or less completely separated (Fig. 5, *e*). Differentiated pericyclic cells then appear on the abaxial side of the sclerenchymatous tissue between the bundles. These are recognisable as lignified tracheidal tissues with young bordered pits. A row of a few regularly arranged cells with dense contents is now formed outside the developing pericyclic tissue. This takes at first the form of four or five cells in the region between the bundles (Fig. 5, *e*), but these rapidly increase in number and spread round the bundles, following the course of the pericyclic tissue previously developed (Fig. 5, *f*). These endodermal cells have most of their radial longitudinal walls lignified when they first become recognisable and suberisation of these walls soon follows. A slight amount of lignification is seen in both the inner and outer tangential walls. Details of the arrangement of the leaf tissues at this stage are given in Fig. 6, which shows the leaf in transverse section.

As the development of the endodermis proceeds, the sclerenchymatous tissue between and around the bundles diminishes in extent and finally disappears. The position in which the endodermis develops last in passing up into the leaf is on the flat adaxial side of this structure, where it is in contact with the other needle of the pair.

It is interesting to find that no completely thickened endodermal layer is present within the sheath of sclerotic scale leaves investing the base of the needles. These scale leaves afford protection for the growing base of the leaf which possesses no thickened hypodermal tissue.

The general structure of the endodermal cell in the leaf of *Pinus sylvestris* shows that these cells must check to a certain extent the passage of water from the xylem to the outer tissues of the leaf. The suberisation of the radial and the transverse walls of the cells probably retards the passage of water outwards through the endodermal walls, only the lignocellulose strip in the centre of the wall being permeable. The transpiration current must thus enter the endodermal cell chiefly by way of the inner tangential wall and pass through the protoplasm to the outer tangential wall. Further, passage of water through the tangential walls is restricted owing to the partial suberisa-

tion of these walls; communication between adjacent endodermal cells is afforded by means of the simple unthickened pits present in the radial walls.

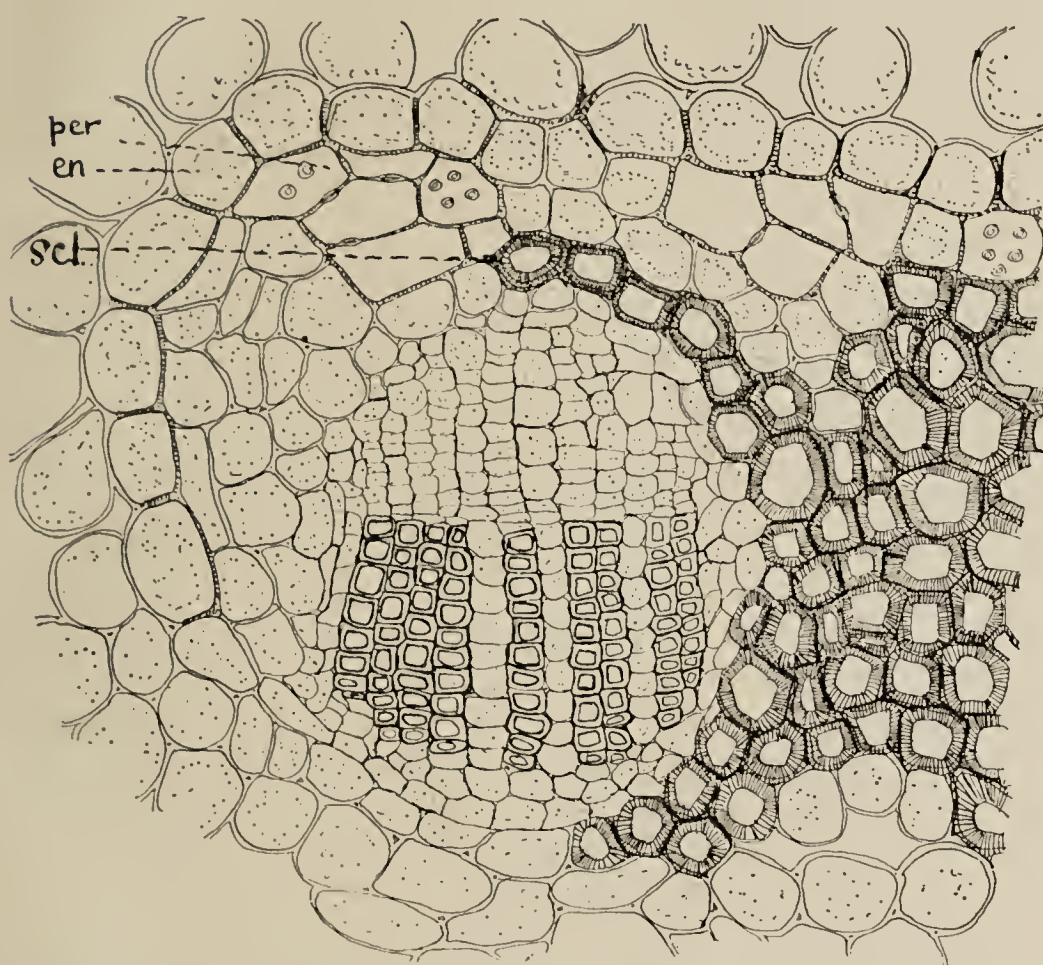


Fig. 6. *Pinus sylvestris* L. Part of a transverse section from the base of a needle showing the developing endodermis and pericycle. *en.*, endodermis; *per.*, pericycle; *scl.*, sclerenchyma. ($\times 240$.)

II. *Pinus laricio* Poir.

The leaves of the Corsican Pine have an endodermis resembling very closely that of *Pinus sylvestris*. The endodermal layer appears very regular in transverse section. The radial walls are suberised and pitted, the suberin layer, as in *Pinus sylvestris*, being deposited on the outside of a central core of lignified material. The inner tangential walls are pitted and consist mainly of lignocellulose with some unthickened cellulose portions. The outer tangential walls are of lignocellulose with patches of suberin on the inner side. The transverse walls resemble in structure the radial walls, but are unpitted. With the exception of the unlignified portions of the inner tangential walls

this leaf exhibits approximately the same degree of xeromorphy as does *Pinus sylvestris*, two or three layers of sclerenchymatous hypodermal tissue being present in this case also.

III. *Pinus excelsa* Lam.

The blue Himalayan Pine has leaves from 5–8 inches long growing in clusters of 2–5. The endodermal cells are rather wider than those of *Pinus sylvestris*, but are shorter when seen in longitudinal section. Their walls are also thinner.

The radial walls have a central lignified core which is suberised on either side and pitted. On treatment with strong sulphuric acid the outer tangential walls become fully carbonised after a time, while the inner tangential walls are only carbonised in certain places. There is less lignification of both tangential walls in this type than is the case with either of the two preceding species, and only those parts of the inner tangential wall are lignified which abut on lignified pericyclic tissue. The transverse walls are lignified, suberised and unpitted.

This leaf is on the whole less xeromorphic in character than that of *Pinus sylvestris* as regards the delicacy of the endodermal cells, the infrequent lignification of the inner tangential wall, and the presence generally of a single layer only of lignified hypodermal tissue. No breaks were found in the continuity of the endodermal sheath in this or in any of the species of *Pinus* investigated.

IV. *Pinus strobus* L.

In the Weymouth Pine the cells of the endodermis are not so regular as is the case in the preceding species and a further reduction in the amount of thickening present is noticeable. The radial and transverse walls have a core of lignified material suberised on either side. Only the former walls are pitted. The outer tangential walls are of lignocellulose, but the inner tangential walls are usually quite unlignified, at most a very slight layer of thickening appearing in a few cells. All the cell walls are more delicate than those of *Pinus excelsa*. A single layer of lignified hypodermal tissue is present. No passage cells were observed in the endodermis.

V. *Picea excelsa* Link.

Daguillon has described the cotyledon of *Picea* and pointed out that, as in *Pinus*, there is a distinct endodermis, but that of *Picea* is distinguished by having bands of thickening on the radial walls. The pericyclic tissue has some thickened elements and some with

bordered pits. The bundle is undivided in the cotyledon, but is usually separated into two in the mature leaf.

A transverse section of the base of an adult leaf shows the endodermis as an irregular layer of cells which cannot be traced continuously round the bundles (Fig. 7). The thickening of the walls of the endodermis varies in character. A few cells were found in which the walls were entirely dissolved by the action of concentrated sulphuric acid.

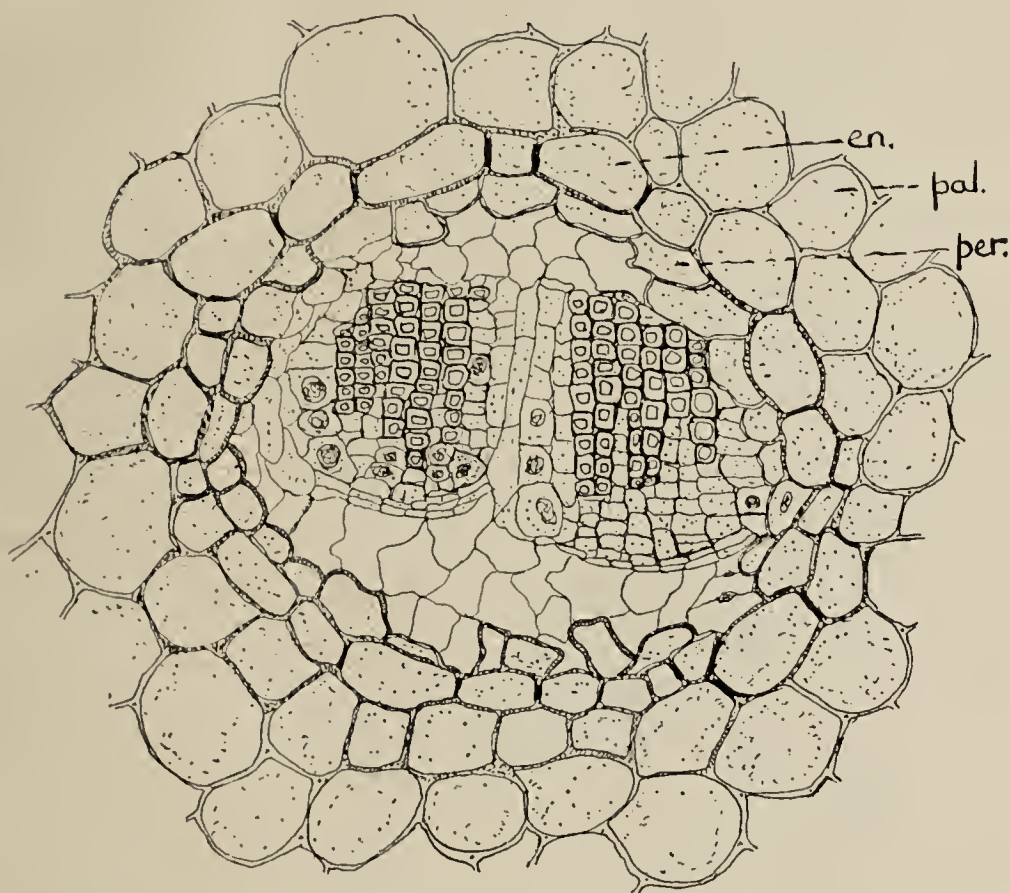


Fig. 7. *Picea excelsa* Link. Transverse section from the basal region of the leaf showing the incomplete endodermis. *en.*, endodermis; *pal.*, palisade tissue; *per.*, pericycle. ($\times 240$.)

The radial walls, in most cases, have a lignified core with suberised layers on the surface and numerous pits. Usually both inner and outer tangential walls are lignified and may in addition be suberised over part of their surfaces.

Towards the middle of the length of the leaf a continuous sheath of thickened endodermal cells is present; this sheath dies out just before the tip of the leaf is reached. The tangential walls are in many cases of cellulose and readily dissolve in sulphuric acid, though parts

of these walls undergo lignification and occasionally suberisation as well. The radial and transverse walls are always both lignified and suberised, the former being pitted. Fig. 8 shows a transverse section taken from about the middle of the leaf. Some of the pericyclic cells have very thick, lignified walls, and a considerable amount of suberin is deposited in the walls of the tracheidal tissue, more than is the



Fig. 8. *Picea excelsa* Link. Transverse section from the middle region of the leaf showing an uninterrupted endodermis. *en.*, endodermis; *s.*, suberin; *lig.*, lignocellulose; *c.*, cellulose. ($\times 240$.)

case in *Pinus*. In *Picea* there is also a greater development of epidermal cuticle and of sclerenchymatous hypodermal tissue than in *Pinus*. This development of relatively impermeable tissue in the cortical region is especially noticeable at the base of the leaf where the endodermal sheath is incomplete. Here from 6–7 layers of sclerenchymatous hypodermal tissue are developed, while towards the middle of the

leaf only 2-3 layers are found, this being the region where no breaks occur in the continuity of the endodermal cells. No passage cells were found in the endodermis above the basal region of the leaf.

VI. *Pseudotsuga Douglasii* Carr.

The cotyledon, as described by Daguillon, possesses a clearly defined endodermis with suberised radial walls and an undivided bundle. In the adult leaf the endodermis shows on the whole less development of thickening than is the case in either *Pinus* or *Picea*. Most of the inner tangential walls dissolve readily in sulphuric acid without undergoing carbonisation, but they are lignified in those parts which abut on lignified pericyclic tissue (Fig. 9, *a* and *b*) as in *Pinus excelsa*. The outer tangential walls are all lignified and suberised, and have pits at long intervals.

In one section examined a single endodermal cell was found to disappear entirely on treatment with sulphuric acid, none of its walls being thickened. This shows that passage cells occur in *Pseudotsuga*, though they appear to be very infrequent.

This leaf is less xeromorphic than that of either *Pinus* or *Picea* in some respects, since only one row of lignified hypodermal tissue at most is present, much of the pericyclic tissue is unligified, and practically no thickened pericyclic cells are present on the abaxial side of the leaf. On the other hand, there is a fair development of cuticle on the epidermis, and the endodermal cells are more completely thickened than is the case in *Picea*.

VII. *Larix europæa* D.C.

Daguillon describes the cotyledon as having an endodermis of large cells thickened on some of the radial walls.

The mature leaf shows very little development of cuticle and also little development of hypodermal schlerenchyma, the latter forming a single layer in the angles of the leaf and in the central region of the upper and lower face. The leaf is dorsiventral to a certain extent, palisade tissue occurring on the upper side and lacunar parenchymatous tissue on the lower. An undivided vascular bundle is usually present, but this may be split near the base of the leaf by a single layer of conjunctive tissue.

The endodermis of the mature leaf has the inner tangential walls unthickened except in a few places where they abut on lignified pericyclic cells (Fig. 9, *c*). The outer tangential walls are lignified and a slight deposit of suberin may be present over part of their surfaces.

The radial and transverse walls have the same structure as in the species previously examined, only the former being pitted and usually at long intervals. On treatment with strong sulphuric acid parts of all the walls of some endodermal cells are dissolved, showing that in

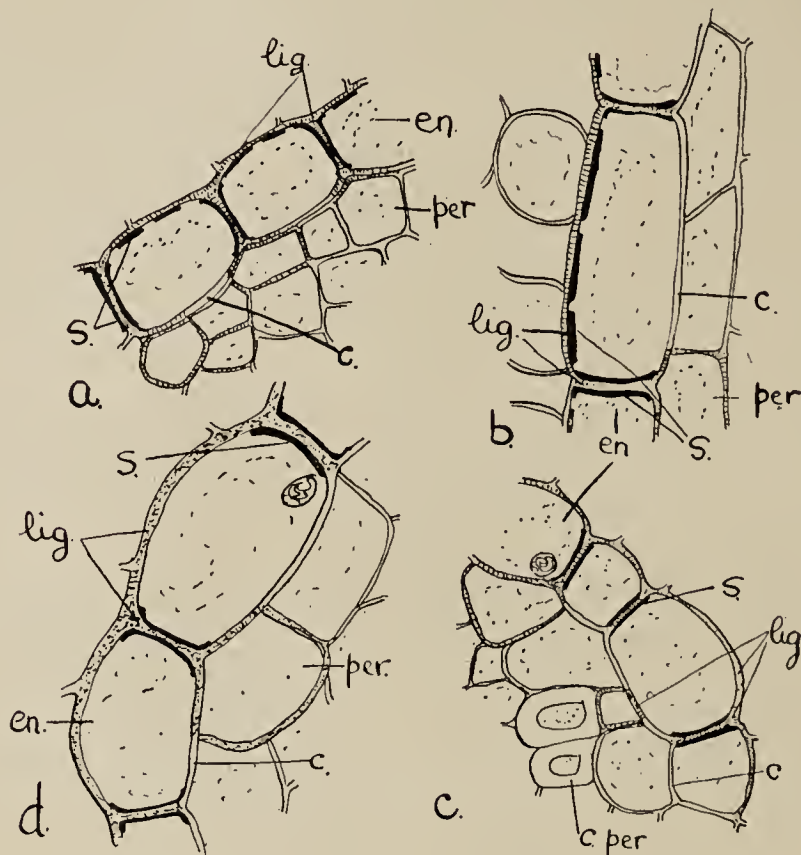


Fig. 9. (a) *Pseudotsuga Douglasii* Carr. Part of a transverse section of the leaf showing portions of the inner tangential walls unlignified. Outer tangential walls lignified, radial walls lignified and suberised.

(b) *Pseudotsuga Douglasii* Carr. Part of a radial longitudinal section of the leaf. The outer tangential walls are lignified and pitted, the inner lignified in places, the transverse walls are lignified and suberised.

(c) *Larix europæa* D.C. Part of a transverse section of a leaf showing the endodermis with portions of the inner tangential walls unlignified.

(d) *Cedrus Libani* Barrel. Part of a transverse section of a leaf showing thick endodermal walls. Inner tangential walls unlignified in places.

en., endodermis; per., pericycle; lig., lignocellulose; s., suberin; c., cellulose; c.per., thick cellulose walled elements of pericycle. ($\times 240$.)

such cases a completely suberised layer is not present, even in the radial walls, and indicating the presence of passage cells.

The endodermal walls are all very delicate, being considerably thinner than those of the species previously described; also the cells are larger in proportion to the size of the leaf. The pericyclic cells are

rarely lignified, some having very thick cellulose walls, especially cells found towards the abaxial side of the leaf.

The xeromorphy of the leaf is, as might be expected for a deciduous tree, less marked than that of the leaves previously described. The suberisation of the radial and transverse walls follows the plan found in the other species investigated with the exception of a few passage cells.

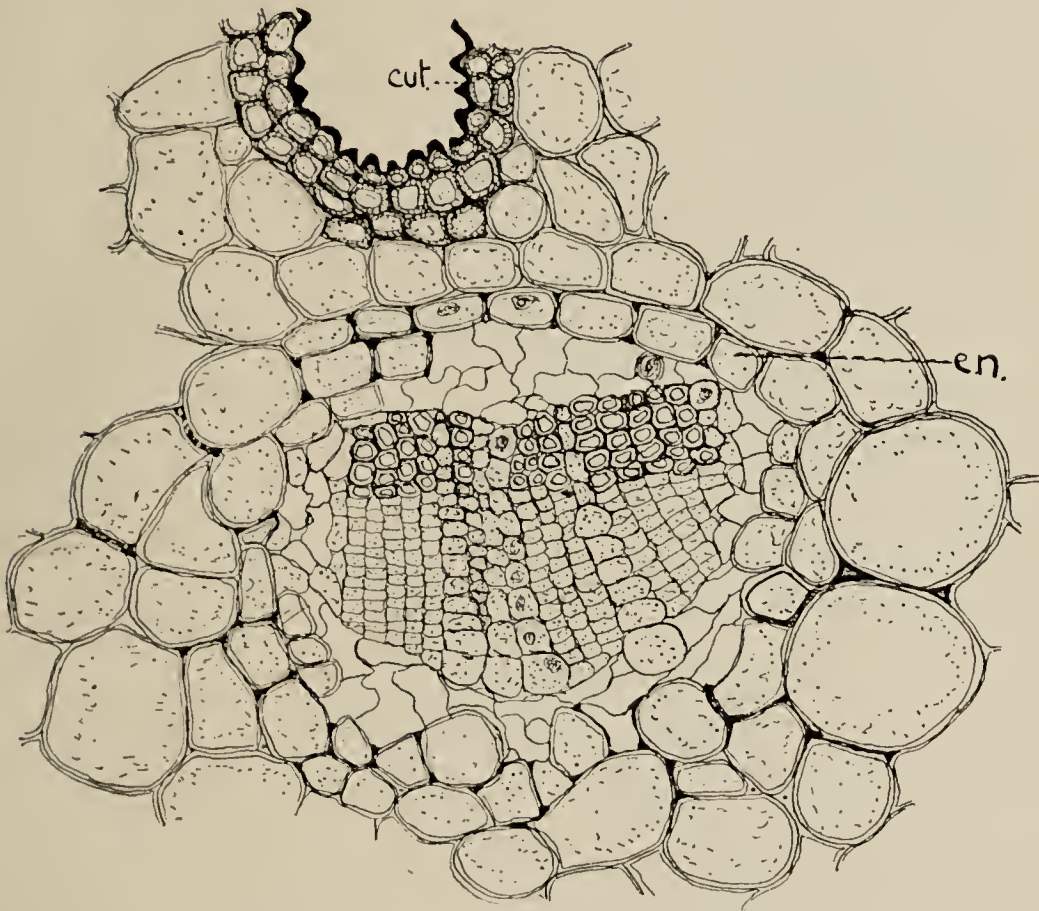


Fig. 10. *Abies pectinata* D.C. Transverse section through the base of the leaf showing the incomplete endodermis. *en.*, endodermis; *cut.*, cuticle. ($\times 240$.)

VIII. *Abies pectinata* D.C.

A certain amount of dorsiventrality can be observed in the leaves of the silver fir, palisade tissue occurring towards the upper surface, and lacunar tissue towards the lower surface of the leaf.

The endodermis resembles that of *Picea excelsa* in its irregularity at the base of the leaf (Fig. 10). As in *Picea* the lack of continuity in the endodermal sheath is accompanied by the greater elaboration of lignified hypodermal tissue in this region, three or four rows of

small cells with lignified walls being present. In the upper parts of the leaf, which have a completely developed endodermis, only two layers of hypodermis are present and the cells are much larger.

Above the basal region of the leaf the endodermis consists of a sheath of rather large cells somewhat irregular in size. The inner tangential walls are mostly of lignocellulose, though here and there an unthickened portion of wall occurs which readily dissolves in

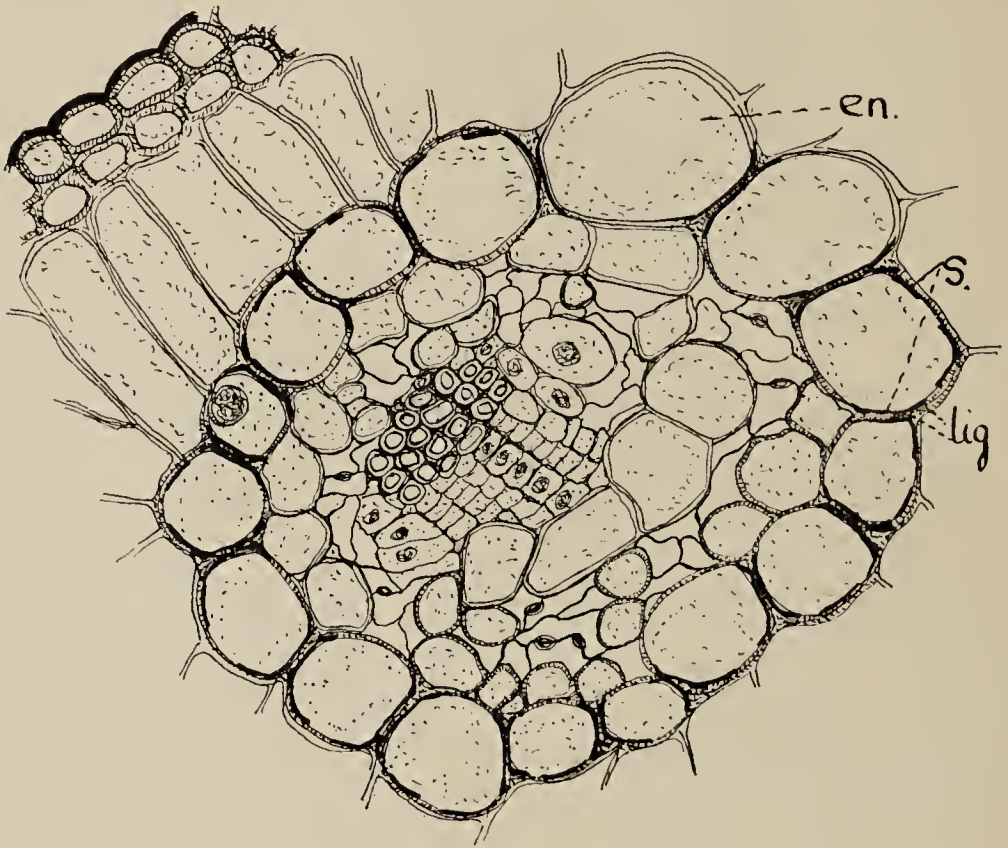


Fig. 11. *Abies pectinata* D.C. Transverse section through middle portion of leaf showing completely developed endodermis. *en.*, endodermis; *s.*, suberin; *lig.*, lignocellulose. ($\times 240$.)

strong sulphuric acid. These cellulose walls are found chiefly in the largest endodermal cells (Fig. 11). The outer tangential walls, with one or two exceptions, are lignified and usually have a thin deposit of suberin on the inner side. The radial walls, as in all the other species described, consist of a central core of lignocellulose bounded on either side by a suberised layer; the radial walls are pitted at long intervals, the pits being most numerous where they adjoin the transverse walls which are unpitted.

IX. *Cedrus Libani* Barrel.

The endodermal layer consists of large regular cells with thick walls, the inner tangential walls being considerably thinner than the others (Fig. 9, *d*). No passage cells were found. Most of the inner tangential walls are unlignified; the outer tangential walls are of lignocellulose and have in addition patches of suberin on the inner surface. The radial and transverse walls consist of a thick inner layer of lignified wall bordered on either side by a suberised layer, and the former are pitted at long intervals.

One to two layers of hypodermal sclerenchyma are present, and the bundle is divided into two by a medullary ray. Numerous pericyclic elements are found with thick cellulose walls, while others are thinner and have lignified walls. Pitted tracheidal cells are also present.

X. *Tsuga canadensis* Carr.

The endodermis of *Tsuga* resembles that of *Abies* in that it consists of large cells rather irregular in size (Fig. 12); the irregularity is more pronounced in *Tsuga*. The continuity of the endodermal sheath is interrupted by a single resin canal in the plane of symmetry. According to Daguillon the resin canal is absent from the cotyledon.

There is on the whole rather slight development of thickening on the tangential walls. Most of the outer and some of the inner are unlignified. Neither tangential wall is suberised. The radial walls are composed of a core of lignocellulose with suberin on the surface. They are pitted at rather long intervals. The transverse walls resemble these in structure but are unpitted. Passage cells are found. The cells surrounding the resin canal are lignified, thus forming with the endodermis a continuous sheath of thickened cells. Only a single layer of hypodermal tissue is present, except by the resin canal, where a double layer occurs. The structure of the leaf is less xeromorphic than is the case for most of the species just described.

XI. *Pseudolarix Kämpferi* Gord.

The cells of the endodermis are very large in proportion to the size of the leaf, and have delicately thin walls resembling in this respect the other deciduous genus *Larix*.

A considerable amount of lignification and suberisation takes place however, none of the walls being entirely soluble in sulphuric acid. Both inner and outer tangential walls are lignified and are suberised in patches. The radial and transverse walls have a thin

core of lignocellulose with a suberised layer on either side. The former are delicately pitted. A considerable number of lignified pericyclic cells are found, a few of these having quite thick walls and resembling the cells with cellulose walls found in the pericycle of *Larix*. A further resemblance to *Larix* is seen in the presence of a single layer of lignified hypodermal tissue.

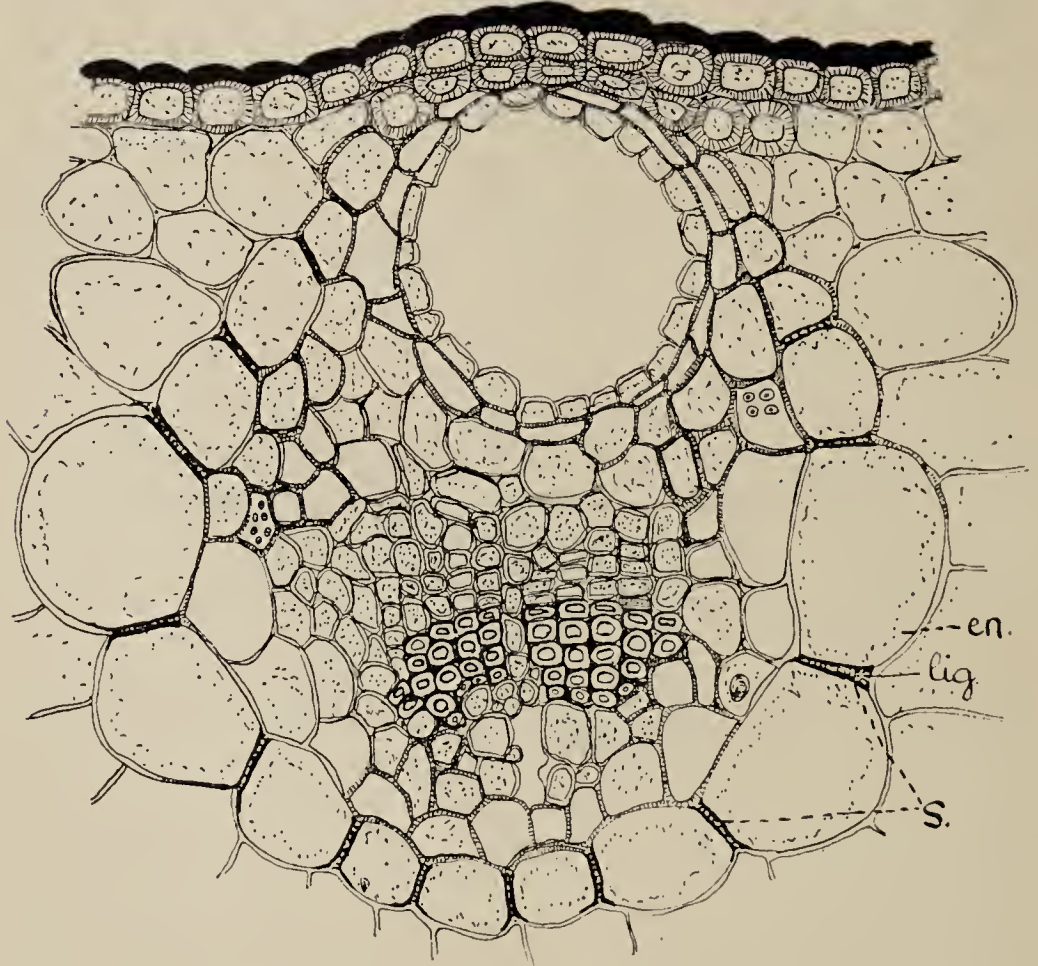


Fig. 12. *Tsuga canadensis* Carr. Transverse section of leaf showing the endodermis interrupted by the single large resin canal. Both inner and outer tangential walls largely unlignified. Radial walls lignified and suberised. *en.*, endodermis; *lig.*, lignocellulose; *s.*, suberin. ($\times 240$.)

Though the walls of the endodermis are so thin, they are all thickened and this leaf seems to be more xeromorphic in character both as regards the endodermis and pericycle than is that of *Larix*.

GENERAL CONCLUSIONS

Eleven species of the Abietineæ were examined and in all of these an endodermis was found to be present which consisted of rather

large cells of four-sided prismatic form with large nuclei and plentiful protoplasmic contents. Starch grains were always present.

Certain constant features are presented by the endodermis in the leaves, among these being the structure of the radial and transverse walls, and it is on this that the consideration of the importance of the endodermis as a physiological unit in the economy of the leaf is based. Caspary's band was not observed to be present, but in every case the radial walls were found to consist of a core of lignocellulose with a layer of suberin superimposed on either side. Simple cellulose pits are always present, though the frequency with which these occur varies in the different genera from many in *Pinus sylvestris* and *Picea excelsa* to few in *Abies pectinata* and *Larix europæa*. The transverse walls consist likewise of a lignified core with surfaces of suberin, but these are never pitted. The continuity of the thickening of the radial walls is interrupted by passage cells in only a very few cases, these being observed in *Tsuga canadensis*, *Pseudotsuga Douglasii* and *Larix europæa*.

The structure of the tangential walls varies considerably in the leaves described. In such species as *Pinus sylvestris* and *Abies pectinata* both tangential walls are usually lignified and have in addition patches of suberised tissue. The amount of suberin deposited seems to depend on the age of the leaf, more being found in older leaves. In other cases, the outer tangential walls only may be lignified, and it is very common to find many of the inner tangential walls wholly of cellulose where they abut on unlignified pericyclic tissue. This is the case in *Pinus excelsa*, *Tsuga canadensis*, *Pseudotsuga Douglasii* and *Larix europæa*. The outer tangential walls were in every case found to be more continuously lignified than the inner ones. Whatever be the nature of the tangential walls, the arrangement of the thickening is always such as to render them permeable to water, the suberised layer, when present, being incomplete. Pits or breaks in the suberin are always present in the most thickened walls.

As has recently been emphasised by Priestley⁽¹²⁾, the passage of water through the walls between the endodermal cells in roots is impossible when an impermeable strip is continuous all round the radial and transverse walls of the cell. This is the case only if at some one point the radial walls are completely suberised throughout their thickness. In all the leaves described the suberin was present in the form of a surface layer on each side of the radial walls, leaving a core of lignocellulose through which water could pass from the

stele to the cortex. The passage of water through these radial walls could also be made impossible if the inner tangential walls possessed an unbroken suberised layer on their outer surfaces, thus preventing the passage of water from the pericyclic tissue into the endodermal cell. This was never found to be the case, suberisation when present occurring on the *inner* layer of the tangential wall. Thus the water stream from the stele to the cortex flowing in response to the pull due to transpiration has two channels through which it may pass. The first is the narrow core of lignocellulose in the centre of the radial walls. The second path is through the tangential walls and the protoplasm of the endodermal cell. It is probable that by far the greater part of the water must travel by the latter path as the suberisation of the radial and the transverse walls is considerable, especially in older leaves. As in the case of roots, control of the rate of the flow of water to the cortex doubtless depends on osmotic phenomena.

Passage of water is possible from one endodermal cell to the next in a tangential direction through the pits in the radial walls, but is impossible in a longitudinal direction as the transverse walls are unpitted.

The relative impermeability of the endodermal walls to water travelling from the conducting tissue to the cortex must have some effect in retarding the transpiration current, and in this lies the importance of the *rôle* of the endodermis as contributor to the xeromorphy of the leaf.

The suberisation of the pericyclic tissue which is found in the more xerophytic types, such as *Pinus sylvestris* and *Picea excelsa*, also helps to reduce the rate of flow of water outwards through the leaf.

An interesting phenomenon is the presence of supplementary checks to the passage of water in those regions of the leaf where the endodermis is incomplete, or where its walls have not yet developed their characteristic thickenings. One example of this is the relatively great development of lignified hypodermal tissue at the base of the leaf in *Picea*, where 6–7 layers of this tissue are found and where a complete endodermal sheath is wanting, whereas, further up the leaf, with the evolution of a continuous endodermis with suberised radial walls, we find the hypodermal tissue is reduced to two layers of cells only. A similar state of affairs can be observed, but in a less marked degree, in *Abies*. Further, in the case of *Pinus*, the base of the leaf, where intercalary growth is proceeding, and where the endodermal

suberisation is not yet developed, is enclosed in a sheath of scale leaves, the cells of which are strongly sclerotic.

It has been shown by Groom(7) that transpiration in coniferous trees is not necessarily slow, and he found that the larch and beech showed the most rapid flow of the transpiration current, the Scots Pine, *Pseudolarix* and *Abies* succeeding them in this respect. The reason for this rapid loss of water by the trees lies in the fact that the aggregate leaf surface is very large, exceeding that of the ordinary dicotylous tree. Correlated with this, the coniferous leaf exhibits the phenomena termed by Prof. Groom "architectural xeromorphism." Histological checks diminish the rate of flow of the transpiration current, and an important factor from this point of view is the suberisation of the radial and transverse endodermal walls. These serve as a barrier delaying the passage of the water stream from the central cylinder to the cortex.

In conclusion I should like to express my thanks to Dr Eric Drabble, at whose suggestion this investigation was undertaken, and to whom I am indebted for helpful criticism during its earlier stages, and to Prof. Dame Helen Gwynne-Vaughan for help and advice during the latter part of its progress. The work was carried out at Birkbeck College, University of London.

SUMMARY

1. The endodermis in the leaves of certain of the Abietineæ has been shown to conform to a general plan, suberisation occurring always on the radial walls which are pitted, and on the transverse walls which are unpitted. The suberin is always deposited as a layer on each side of a lignocellulose core.

2. The structure of the tangential walls varies in the different species described. They are often both lignified and suberised, but sometimes they, especially the inner tangential walls, consist of cellulose. Where these walls are suberised pits or breaks in the suberised membrane are present.

3. In young leaves of *Pinus sylvestris* the development of the endodermal walls proceeds as follows: (1) cellulose, (2) lignocellulose, (3) lignocellulose with a surface layer of suberin. Thickening develops first in the radial walls.

4. When the endodermal sheath is incomplete additional protective tissues are often found. Thus the growing leaf base of *Pinus* is protected by sclerotic scales, and the basal region of the leaf in *Picea* and *Abies* by additional layers of lignified hypoderma.

5. The suberisation and lignification of the radial walls renders the endodermis relatively impervious to the passage of water through the walls alone. Thus the transpiration current must flow largely through the endodermal cell, and it is probable that the protoplasm exerts some control over the rate of flow.

BIBLIOGRAPHY

- (1) CHURCH, A. H. Elementary notes on Conifers. *Oxford Botanical Memoirs*, No. 8.
- (2) ——— Form-Factors in Coniferæ. *Ibid.* No. 9.
- (3) DAGUILLON, A. Observations sur la structure des feuilles de quelques Conifères. *Bull. Soc. Bot. France*, t. 35. 1888.
- (4) ——— Sur le polymorphisme foliaire des Abiétinées. *Comptes Rendus*. 1889.
- (5) ——— Recherches morphologiques sur les feuilles des Conifères. *Revue Gén. Bot.* 11. 1890.
- (6) DE BARY, A. *Comparative Anatomy of the Vegetative Organs of Phanerogams and Ferns*.
- (7) GROOM, P. Remarks on the Œcology of the Coniferæ. *Annals of Botany*, 24. 1910.
- (8) HAAS, P. and HILL, T. G. *An Introduction into the Chemistry of Plant Products*. Longmans, Green & Co. 1910.
- (9) HABERLANDT, G. *Physiological Plant Anatomy*, translated by Montague Drummond. Macmillan & Co. 1914.
- (10) JEFFREY, E. C. The Comparative Anatomy of the Coniferales. II. The Abietinæ. *Mem. Boston Soc. Nat. Hist.* 6. 1904.
- (11) LEE, A. B. The Microtometist's Vade-Mecum. Churchill. 1905.
- (12) PRIESTLEY, J. H. The Mechanism of Root Pressure. *New Phytologist*, 19. 1920.
- (13) ——— Suberin and Cutin. *New Phytologist*, 20. 1921.
- (14) ZIMMERMANN, A. *Botanical Microtechnique*. Translated by J. E. Humphrey. Holt & Co.

THE ORIGIN OF THE HYBRID *PRIMULA*
ELATIOR × *VULGARIS* DEMONSTRATED
 EXPERIMENTALLY IN THE FIELD, WITH
 NOTES ON OTHER BRITISH *PRIMULA*
 HYBRIDS

BY MILLER CHRISTY, F.L.S.

SOON after the appearance, in 1897, of my paper on "*Primula elatior* in Britain¹," I had the pleasure of showing to Dr Bateson, Miss E. R. Saunders, and others the various *Primula* hybrids I had described therein—namely: (1) *P. elatior* × *vulgaris*, (2) *P. elatior* × *veris*, and (3) *P. veris* × *vulgaris*—all growing in a wild state in certain woods in Essex, Suffolk, and Cambridgeshire.

On this occasion Dr Bateson (without disputing my assumption that the plants in question really were hybrids) pointed out that, for purposes of absolute scientific accuracy, their hybrid origin, even if practically certain inferentially, ought to be demonstrated incontestably by means of definite experiment.

Thereupon it was decided to attempt to accomplish this by planting some Oxlips (*P. elatior*) in woods wherein Primroses (*P. vulgaris*) only were growing and some Primroses in other woods wherein Oxlips only were growing, and then waiting to see whether plants similar to those I had described as hybrids between these two species would appear naturally in the course of time.

That the plants I had described as hybrids were really such might, of course, have been demonstrated much more easily and quickly by artificially pollinating flowers of either of the two reputed parents with pollen from flowers of the other on covered plants growing in cultivation and then raising plants from any fertile seed resulting. As a matter of fact, Miss Saunders succeeded in doing this soon after the time when our experiments described herein were begun², and since then Mr E. G. Highfield has done the

¹ *Journ. Linn. Soc., Bot.* 33 (1897), pp. 172–201.

² Miss Saunders writes me: "In 1899 and 1900, I raised a number of hybrids between *P. elatior* and *P. vulgaris* from crosses which I made for the purpose of testing the views expressed in your original paper. The results entirely confirmed your conclusions; but, having done this and having found the sterility so great that there was no likelihood of obtaining any genuine ratios in support of the Mendelian Theory or otherwise, I did not continue the experiments and no record was published."

same¹. Obviously, however, it would be doubly satisfactory to have the confirmatory evidence of experiments carried out actually in the field². The chief difficulty in following the latter (natural) method lies in the very long period it is necessary to allow when experimenting with the two species named; for both grow in woods and both flower fully only when these woods are cut down, which is done only at intervals of from twelve to fifteen years; so that it is necessary to wait at least that time before the result of such an experiment can become apparent.

The hybrid in question (namely, *P. elatior* × *vulgaris*) is little known to British botanists, though it was long since met with and definitely recognised upon the Continent. It is apparently the *P. media* of Petermann, the *P. digena* of Kerner³, the *P. elatiori-officinalis* of various writers, and has also been known under other names; but the synonymy of all the three *Primula* hybrids mentioned herein is confused absolutely beyond hope of disentanglement. The first to call attention to its occurrence in this country was, I believe, the late G. S. Gibson, of Saffron-Walden⁴. Later, I myself pointed out more fully the facts as to its occurrence in Britain⁵. I showed that it occurs abundantly round the margins of the two Oxlip "Districts" defined by me. Here alone the Oxlip comes in contact with the Primrose, which grows in profusion all round, but (for a reason I have attempted to explain elsewhere⁶) *does not grow within these Districts*. It is the only one of the three *Primula* hybrids which is ever to be seen in abundance at any one spot⁷.

¹ See *Knowledge*, 39 (1916), pp. 113-117.

² I had long been familiar with the fact that hybrids between the two species named arise spontaneously in gardens in which Oxlip plants have been introduced among a number of Primrose plants. Thus, in May 1883, I sowed some Oxlip seed in a garden at Chignal St James, near Chelmsford, in which there were many Primrose plants. It produced nineteen plants; and, within a year or two, obvious hybrids between the two species appeared. Some Oxlip plants were still growing in the garden nearly a quarter of a century later (on 13th April 1906), together with some of the hybrids. Again, in April 1884, I saw in the garden of the late Mr James H. Tuke, at Bancroft, Hitchin, in which there were growing both Oxlips and Primroses, a pink-flowered Oxlip, evidently a hybrid with some red-flowered Primrose. A red-flowered garden form of the Oxlip, no doubt of similar hybrid origin, is mentioned by Pax and Knuth (*Primulaceae*, in Engler's *Pflanzenreich*, 22 (1905), p. 50).

³ *Oest. bot. Zeit.* (1875), p. 79.

⁴ *Phytologist*, 1 (1844), p. 996.

⁵ *Trans. Essex Field Club*, 3 (1884), pp. 204-205, and *Journ. Linn. Soc., Bot.* 33 (1897), pp. 193-197.

⁶ See *Journ. of Ecology* (1922), pp. 209-210.

⁷ The number of plants existing in Britain of another hybrid noticed hereafter (namely, *P. veris* × *vulgaris*) is probably actually greater, as this occurs all over the country, but the number of plants to be met with at any one spot is always small (see *post*, p. 299).

This hybrid sometimes produces single (scapeless) flowers like those of the Primrose—a fact I observed as long ago as 1880¹. These single (hybrid) flowers must not be confused, however, with the very similar single flowers which are produced not uncommonly by the (pure-bred) Oxlip, but *always very early in the flowering-season* and on plants which, later in the season, produce also flowers in umbels, as usual². These latter single flowers are certainly not of hybrid origin and are due solely to the suppression of the peduncle.

I have observed that, in cases in which hybrid plants produce these single flowers, the tendency to produce them very early in the flowering-season is *specially* marked—owing, no doubt, to the influence of the Primrose strain in them; for the Primrose flowers very early. Thus, in the spring of 1898, I had in cultivation, in a cold greenhouse, a hybrid plant which had produced, the year before, both single and umbellate flowers. About the 20th February (the time when the Primrose might be expected to flower in such circumstances), it produced several single flowers. By the 9th April (about the time when the Oxlip might be expected to flower), it had produced two umbels of the usual hybrid type—one fully out: the other half out. In the following year (1899), the same plant (or, possibly, another; for I had several) began producing single flowers about the same time (say 20th February) and had produced altogether fifteen or twenty such by the 25th March; on which date, the umbels on the same plant were just beginning to grow.

Our experiments made in order to demonstrate, in the field, the hybrid origin of this plant were as follows:

Experiment I. As a first step, a number of Primrose plants (some long-styled, some short-styled), obtained by me from a wood near Chelmsford (which is about eleven miles from the nearest point on the margin of the easternmost of the two Oxlip “Districts” defined by me), were planted by Miss Saunders, in the presence of Dr Bateson, on 1st November 1898, in nine separate patches, in different parts of Peverell’s Wood, near Saffron-Walden, a wood in which Oxlips grow in immense abundance. The wood is at least two miles

¹ See *Trans. Essex Field Club*, 3 (1884), pp. 186–190.

² The occurrence of such single flowers on the Oxlip near Leipzig was long since recorded by Petermann (*Fl. Lipsiensis*, p. 170: 1838). Their early appearance is, doubtless, evidence that ancestrally the Oxlip bore acaulescent flowers.

within the said Oxlip area, so that the Primrose plants in question were at least that distance from any other Primrose plants¹.

The patches were inspected from time to time in succeeding years; and, when they could be discovered (the wood being grown up), the plants were found to be alive and flowering. Later, some of the patches appeared to have died out—smothered, doubtless, by the dense over-growth. I visited the wood early in April 1915, when I found that the plants forming one patch were in flower sparingly, but I could see no trace of any hybrids having arisen. About a fortnight later, however, on 30th April, Miss Saunders found that, not only were some of the original Primrose plants alive and in flower, but that, within the areas of four of the original nine patches, other plants, obviously hybrids (*P. elatior* × *vulgaris*) and of the exact type of those I had described originally as this hybrid, had come into existence and were also in flower². In the circumstances explained, these latter plants can have come into existence only as a result of natural hybridisation between the few introduced Primrose plants and the countless Oxlip plants growing all around them.

Thus, after nearly seventeen years, our experiment proved perfectly successful and completely demonstrated all we had sought to demonstrate.

Experiment II. On 4th February 1906, I planted in Bush Wood, Broomfield, near Chelmsford, nearly fifty Oxlip roots (including plants of both forms) obtained from near Saffron-Walden; and, on the 11th, I planted about thirty more, obtained from the same place, in College Wood, about a mile distant, both woods containing a profusion of Primroses and being at least ten miles distant from the nearest point on the margin of the eastern Oxlip District³. Both woods had recently been cut down. It was obvious, therefore, that some fifteen years would have to elapse before any result of the experiment could become apparent.

In various succeeding years I endeavoured to find some of these plants; but, both woods being grown up, I was able to find one or

¹ Conceivably there may have been a few Primrose plants under cultivation in cottage gardens within a mile; but, if so, they were so few that they were hardly likely to affect the outcome of our experiment.

² The plants in another patch were not yet in flower, and the four remaining patches could not be found.

³ In each case, I planted the plants, not in patches, but in three straight lines extending right across the wood. This ensures that, if one can find only two plants in a row, one can easily find all the rest; which is often difficult when the plants are set out haphazard.

two only. On 4th May 1922, I searched both woods carefully. College Wood had, I found, never been cut since I planted the Oxlips in it¹; and (probably as a result of this) I was unable to discover either the Oxlips I had planted or any hybrids arising from them. Bush Wood had, I found, been cut a year or two before and had grown up again; with the result that here also I was able to discover neither my Oxlip plants nor hybrids due to them.

This Experiment has, therefore, failed—at all events, so far.

Experiment III. In January 1906, on the suggestion of Mr G. A. Boulenger, F.R.S., I sent a number of Primrose roots, obtained near Chelmsford (where no Oxlips grow), to Maredsous, in the Belgian Ardennes². These Primrose roots were carefully planted by the Rev. Dom Beda Lebbe, O.S.B., of the great Abbaye there, on a wooded hillside near and belonging to the Abbaye, there being no Primroses growing naturally in that part of Belgium, but an abundance of Oxlips in the woods and damp meadows all around.

I saw the plants in April 1910, when they appeared to be flourishing and were flowering freely. Later, however, they languished; and Mr Boulenger, who has visited the spot recently, informs me that most or all of them have now died out, without producing any hybrids³. I fancy the position in which they were planted was not quite suited to them.

Thus this Experiment failed completely—not in itself, but through a subsidiary cause.

Nevertheless, the fact that the Oxlip and the Primrose hybridise freely in the wild state when growing in close proximity has now been demonstrated incontestibly by actual experiment in the field.

Nor is this at all surprising; for the two species are, though quite distinct, so closely allied that Linnæus and most of the earlier botanists regarded them as mere varieties of one variable species. Moreover, both flourish in much the same habitats (usually woods, but sometimes open meadows) and it is known that both are visited and pollinated by the same species of insects—a matter I have

¹ Many woods in the Eastern Counties have remained uncut several years beyond the usual time for cutting them, owing to the War, the scarcity and dearness of labour, and the small value of brushwood recently.

² I had infinite trouble to get them through the Belgian Customs, owing to a fear that they might convey the Phylloxera vine disease, which does not attack Primroses, from England, where it did not exist, to Belgium, where there are no vineyards!

³ See also his record of this fact in *Comptes Rendus de l'Acad. des Sciences*, 170 (Paris, 1920), p. 1298 n.

discussed fully elsewhere¹. Thus, in early spring, I have myself seen the common Brimstone butterfly visit, not only these two species, but also the Cowslip. In Germany, Knuth saw it visit all these three species on one single day². Again, all three species are visited more or less commonly by various species of Hymenoptera and Diptera having tongues long enough to effect their pollination and, therefore, to hybridise them.

Inasmuch as it has been proved by actual experiment in the field that hybrids between the Oxlip and the Primrose (namely, *P. elatior* × *vulgaris*) do arise spontaneously in a state of nature, it is a reasonable conclusion that the second of the three *Primula* hybrids mentioned at the outset (namely, *P. elatior* × *veris*) also arises in the same way.

This is a very rare hybrid, occurring sporadically only: never abundantly anywhere. I doubt if I have seen as many as forty plants of it in the forty years during which I have made a study of our British *Primulas*. This rarity is somewhat surprising in view of the fact that the Cowslip (unlike the Primrose) grows not uncommonly throughout the whole of the Oxlip Area defined by me. The rarity of this hybrid is probably due to the fact that the flowering-times of its two parent species differ somewhat (the middle of the Cowslip flowering period being from two to three weeks later than that of the Oxlip, though the flowering periods of the two overlap) and that their habitats also differ (the Oxlip growing mainly in woods: the Cowslip in meadows); so that there is comparatively little probability of hybridisation between them³. The hybrid is in every way intermediate, in all its characters, between its two parent species. So far as I have observed, single (non-pedunculate) flowers never occur in this hybrid, its flowers being always borne in umbels—doubtless because both its parents normally have umbellate inflorescences. As I have seen the hybrid growing in both woods and meadows, it seems probable that the female parent may be supplied by either species.

On the same grounds, it is a reasonable assumption that the third of the three *Primula* hybrids mentioned at the outset (namely, *P. veris* × *vulgaris*) also arises in the same way.

¹ See *Journ. Linn. Soc., Bot.* 46 (1922), pp. 105–139.

² *Flower Pollination*, 2 (1899), p. 69.

³ Kerner says, however (*Nat. Hist. of Plants*, 2 (1902), p. 404), that “artificial pollination between them only occasionally leads to any result.”

Nearly every European botanist must be acquainted with this curious plant; for it occurs in many parts of Europe and in all parts of Britain, but everywhere more-or-less sporadically and nowhere abundantly. It has been known by a host of names, such as *Primula variabilis*, *P. caulescens*, *P. intricata*, *P. vulgaris* var. *multiflora*, var. *caulescens*, var. *variabilis*, and the like. In Britain it is often spoken of popularly as the "False Oxlip," or the "Hybrid Oxlip." Here it is still often confused with the "True" or "Bardfield" Oxlip (*P. elatior* Jacquin), though its distinctness was pointed out by Henry Doubleday as long ago as 1842. In 1897 I clearly defined the distribution of the latter in this country¹.

The "Hybrid Oxlip" is an exceedingly-variable plant. Its flowers (which are always more or less sterile) present almost every conceivable intermediate gradation between those of the two parent species, in respect of colour, size, and general floral characters. They are sometimes borne singly (without any obvious peduncle); sometimes in more-or-less lax straggling umbels; and sometimes both forms of inflorescence appear on the same plant. Its hybrid nature was early suspected on the Continent. As long ago as 1843, Godron expressed his belief that it was a hybrid²; and, in the following year, he declared himself convinced that it was so³. Nearly twenty years later, he discussed the question exhaustively⁴, maintaining his conclusion and even declaring that he had produced hybrids by artificial cross-pollination of the two reputed parents, though he gives no details of this experiment. Soon after, in 1868, Darwin accepted this view completely and set forth at length his reasons for so doing⁵. Later, in 1897, I summarised the known facts in favour of this view, which I accepted unreservedly⁶. A large majority of the many French and British writers who have discussed the plant recently have done the same⁷. It is, in spite of its partial sterility, a much handsomer and more vigorous plant than either of its parents, as is so often the case with first-cross hybrids. For this reason, cottagers

¹ See *Journ. Linn. Soc., Bot.* **33** (1897), pp. 183-188.

² *Fl. de Lorraine*, **2** (1843), p. 225.

³ *De l'hybridité dans les Végétaux*, p. 21 (Nancy, 1844).

⁴ *Bull. Soc. Bot. de France*, **10** (1863), pp. 178-182.

⁵ See *Journ. Linn. Soc., Bot.* **10** (1869), pp. 437 and 443-449; also *Different Forms of Flowers*, etc. pp. 55-71 (1877).

⁶ *Journ. Linn. Soc., Bot.* **33** (1897), pp. 174-176.

⁷ Among the latest are E. G. Highfield (see *ante*, p. 294 n.) and Blaringhem, in *Compt. Rend. de l'Acad. des Sci.* **172** (1921), pp. 992-994. The last-named writer even suggests that the Primrose itself may be a hybrid, because (he says) a considerable proportion of its pollen is always more or less imperfect—a statement which requires to be confirmed.

very frequently dig up roots and remove them to their gardens, thus causing it to be much less common than it would be otherwise.

Again, in connection with this hybrid, there is nothing surprising in hybrids between the two parent species arising naturally wherever the two grow in close proximity, as they often do. It is true that their habitats differ somewhat (the Primrose growing mainly in woods and hedge-rows: the Cowslip in open meadows¹); but Cowslips often grow just within the edges of a wood in which Primroses grow, or in meadows closely adjoining, and the hybrid is found not infrequently in these situations. Moreover, it is known that both the parent species are visited by the same species of insect². Thus, as stated already³, the Brimstone butterfly (*Gonepteryx rhamni*) has been seen to visit both, as also have several large species of bee. On 13th April 1914, I watched a small blackish bee (*Anthophora acervorum*) visiting Primroses on the edge of a cut-down wood at Roxwell, Essex. After it had visited twenty Primrose flowers, it immediately visited two Cowslip flowers growing only two or three feet distant and on (or very close to) a spot where, in earlier years, I had seen undoubted hybrids growing—an observation very much to the point in this connection.

¹ Inasmuch as hybrids are found not uncommonly in both these habitats, it seems probable that both plants supply at times the female parent.

² See *Journ. Linn. Soc., Bot.* 46 (1922), pp. 105–139.

³ See *ante* p. 298.

